

## ENVIRONMENTAL PROTECTION AGENCY

### 40 CFR Part 136

[FRL-     ]

The Administrator signed the following rule on November 8, 2002, and EPA is submitting it for publication in the *Federal Register*. While we have taken steps to ensure the accuracy of this pre-publication version of the rule, it is not the official version for purposes of compliance. Please refer to the official version in a forthcoming *Federal Register* publication, on GPO's website at: [http://www.access.gpo.gov/su\\_docs/aces/aces140.html](http://www.access.gpo.gov/su_docs/aces/aces140.html), or on EPA's website at <http://www.epa.gov/waterscience/WET>.

### **Guidelines Establishing Test Procedures for the Analysis of Pollutants; Whole Effluent Toxicity Test Methods; Final Rule**

**AGENCY:** Environmental Protection Agency (EPA).

**ACTION:** Final Rule.

**SUMMARY:** In this final regulation, EPA ratifies approval of several test procedures for measuring the toxicity of effluents and receiving waters. The test procedures are commonly referred to as whole effluent toxicity or WET test methods. EPA also withdraws two WET test methods from the list of nationally-approved biological test procedures for the analysis of pollutants. This action also revises some of the WET test methods to improve performance and increase confidence in the reliability of the results. Today's action will satisfy settlement agreement obligations designed to resolve litigation over an earlier rulemaking that originally approved WET test methods.

**DATES:** This regulation is effective [insert 30 days from date of publication in the *Federal Register*]. For judicial review purposes, this final rule is promulgated as of 1:00 p.m. Eastern Standard Time on [insert 14 days from date of publication in the *Federal Register*] in accordance with 40 CFR 23.7. The incorporation by reference of certain publications listed in

this rule is approved by the Director of the Federal Register as of **[insert 30 days from date of publication in the Federal Register]**.

**FOR FURTHER INFORMATION CONTACT:** Marion Kelly; Engineering and Analysis Division (4303T); Office of Science and Technology; Office of Water, U.S. Environmental Protection Agency; Ariel Rios Building; 1200 Pennsylvania Avenue, NW; Washington, DC 20460, or call (202) 566-1045, or E-mail at [kelly.marion@epa.gov](mailto:kelly.marion@epa.gov). For technical information regarding method changes in today's rule, contact Debra L. Denton, USEPA Region 9, c/o SWRCB, 1001 I Street, Sacramento, CA 95814, or call (916)341-5520, or E-mail [denton.debra@epa.gov](mailto:denton.debra@epa.gov).

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### I. General Information

#### A. *Potentially Regulated Entities*

EPA Regions, as well as States, Territories, and Tribes authorized to implement the National Pollutant Discharge Elimination System (NPDES) program, issue permits that comply with the technology-based and water quality-based requirements of the Clean Water Act. In doing so, NPDES permitting authorities make a number of discretionary choices associated with permit writing, including the selection of pollutants to be measured and, in many cases, limits for those pollutants in permits. If EPA has “approved” (i.e., promulgated through rulemaking) standardized test procedures for a given pollutant, the NPDES permitting authority must specify one of the approved testing procedures or an EPA-approved alternate test procedure for the measurements required under the permit. In addition, when a State, Territory, or authorized Tribe provides certification of Federal licenses under Clean Water Act section 401, States, Territories and Tribes are directed to use the approved testing procedures. Categories and entities that may be regulated include:

Category	Examples of potentially regulated entities
Federal, State, Territorial, and Indian Tribal Governments	Federal, State, Territorial, and Tribal entities authorized to administer the NPDES permitting program; Federal, State, Territorial, and Tribal entities providing certification under Clean Water Act section 401
Municipalities	Municipal operators of NPDES facilities required to monitor whole effluent toxicity
Industry	Private operators of NPDES facilities required to monitor whole effluent toxicity

This table is not intended to be exhaustive, but rather provides a guide for readers regarding entities likely to be regulated by this action. This table lists the types of entities that EPA is now aware could potentially be regulated by this action. Other types of entities not listed in the table could also be regulated. To determine whether your facility or organization is regulated by this action you should carefully examine 40 CFR 122.41(j)(4), 122.44(i)(1)(iv), and 122.21. If you have questions regarding the applicability of this action to a particular entity, consult the first person listed in the preceding "FOR FURTHER INFORMATION CONTACT" section.

*B. How Can I Get Copies Of Related Information?*

1. Docket

EPA has established an official public docket for this action under Docket ID No. WET-X (Electronic Docket No. OW-2002-0024). The official public docket consists of the documents specifically referenced in this action, any public comments received, and other information related to this action. Although a part of the official docket, the public docket does not include Confidential Business Information (CBI) or other information whose disclosure is restricted by

statute. The official public docket is the collection of materials that is available for public viewing at the Office of Water (OW) Docket, in the EPA Docket Center (EPA/DC), EPA West, Room B-102, 1301 Constitution Avenue N.W., Washington, D.C. The EPA Docket Center Public Reading Room is open from 8:30 a.m. to 4:30 p.m. EST, Monday through Friday, excluding legal holidays. The telephone number for the Public Reading Room is (202) 566-1744, and the telephone number for the OW Docket is (202) 566-2426.

## 2. Electronic Access

You may access this Federal Register document electronically through the EPA Internet under the “Federal Register” listings at <http://www.epa.gov/fedrgstr/>.

An electronic version of the public docket is available through EPA’s electronic public docket and comment system, EPA Dockets. You may use EPA Dockets at <http://www.epa.gov/edocket/> to view public comments, access the index listing of the contents of the official public docket, and to access those documents in the public docket that are available electronically. Although not all docket materials may be available electronically, you may still access any of the publicly available docket materials through the docket facility identified in Unit I.B.1. Once in the system, select “search,” then key in the appropriate docket identification number.

## II. Statutory Authority

EPA promulgates today's rule pursuant to the authority of sections 301, 304(h), 402, and 501(a) of the Clean Water Act (“CWA” or the “Act”), 33 U.S.C. 1311, 1314(h), 1342, 1361(a) (the “Act”). Section 101(a) of the Act sets forth the “goal of restoring and maintaining the chemical, physical, and biological integrity of the nation’s waters” and prohibits “the discharge of toxic pollutants in toxic amounts.” Section 301 of the Act prohibits the discharge of any pollutant

into navigable waters unless the discharge complies with a National Pollutant Discharge Elimination System (NPDES) permit, issued under section 402 of the Act. Section 304(h) of the Act requires the Administrator of the EPA to "promulgate guidelines establishing test procedures for the analysis of pollutants that shall include the factors which must be provided in any certification pursuant to section 401 of this Act or permit applications pursuant to section 402 of this Act." Section 501(a) of the Act authorizes the Administrator to "prescribe such regulations as are necessary to carry out his function under this Act." EPA publishes CWA analytical method regulations at 40 CFR Part 136. The Administrator also has made these test procedures applicable to monitoring and reporting of NPDES permits (40 CFR Parts 122, §§122.21, 122.41, 122.44, and 123.25), and implementation of the pretreatment standards issued under section 307 of the Act (40 CFR Part 403, §§403.10 and 403.12).

### **III. Background**

#### *A. Regulatory History*

On October 16, 1995, EPA amended the "Guidelines Establishing Test Procedures for the Analysis of Pollutants," 40 CFR Part 136, to add a series of standardized toxicity test methods to the list of Agency approved methods for conducting required testing of aqueous samples under the CWA (60 FR 53529) (WET final rule). The WET final rule amended 40 CFR 136.3 (Tables IA and II) by adding acute toxicity methods and short-term methods for estimating chronic toxicity. These methods measure the toxicity of effluents and receiving waters to freshwater, marine, and estuarine organisms. Acute methods (USEPA, 1993) generally use death of some percentage of the test organisms during 24 to 96 hour exposure durations as the measured effect of an effluent or receiving water. The short-term methods for estimating chronic toxicity (USEPA, 1994a; USEPA, 1994b) use longer durations of exposure (up to nine days) to ascertain



the adverse effects of an effluent or receiving water on survival, growth, and/or reproduction of the organisms. The methods listed at 40 CFR Part 136 for measuring aquatic toxicity are referred to collectively as “WET test methods,” methods specific to measuring acute toxicity are referred to as “acute” test methods, and short-term methods for estimating chronic toxicity are referred to as “chronic” methods.

EPA standardized the test procedures for conducting the approved acute and chronic WET test methods in the following three method manuals, which were incorporated by reference in the WET final rule: *Methods for Measuring the Acute Toxicity of Effluents and Receiving Water to Freshwater and Marine Organisms*, Fourth Edition, August 1993, EPA/600/4-90/027F (acute method manual); *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms*, Third Edition, July 1994, EPA/600/4-91/002 (freshwater chronic method manual); and *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Marine and Estuarine Organisms*, Second Edition, July 1994, EPA/600/4-91/003 (marine chronic method manual). EPA explains in the *Technical Support Document for Water Quality-Based Toxics Control* (TSD) (USEPA, 1991) that these WET test methods, along with chemical controls and bioassessments, are a component of EPA’s integrated strategy for water quality-based toxics control. The TSD recommends that WET tests using the most sensitive of at least three test species from different phyla be used for monitoring the toxicity of effluents.

Since the 1995 WET final rule, EPA has issued several rulemakings and guidance documents in fulfillment of settlement agreements to resolve judicial challenges to the WET final rule (see Settlement Agreement discussion in Section III.B). On February 2, 1999, EPA published technical corrections that incorporated into the WET final rule an errata document to correct

minor errors and omissions, provide clarification, and establish consistency among the WET final rule and method manuals (64 FR 4975; February 2, 1999). On July 18, 2000, EPA announced the availability of a WET Variability Guidance Document (65 FR 44528; July 18, 2000). On July 28, 2000, EPA published the availability of a WET Method Guidance Document (65 FR 46457; July 28, 2000). On September 28, 2001, EPA proposed specific revisions to the WET test methods, and EPA proposed to ratify its previous approval of these methods (66 FR 49794; September 28, 2001) (see Section III.C). Today, EPA takes final action on the September 2001 proposal.

### *B. Settlement Agreement*

Following promulgation of the WET methods on October 16, 1995, several parties challenged the rulemaking (Edison Electric Institute v. EPA, No. 96-1062 (D.C. Cir.); Western Coalition of Arid States v. EPA, No. 96-1124 (D.C. Cir.); and Lone Star Steel Co. v. EPA, No. 96-1157 (D.C. Cir.)). To resolve the litigation, EPA entered into settlement agreements with the various parties and agreed to publish a technical correction notice, publish a method guidance document and a variability guidance document, conduct an interlaboratory variability study, publish a peer-reviewed interlaboratory variability study report (including a table of coefficients of variation), address pathogen contamination, propose specific technical method changes, and propose to ratify or withdraw WET test methods evaluated in the interlaboratory variability study. Today's final action fulfills EPA's obligations under the settlement agreements.

### *C. Proposed Rule*

On September 28, 2001, EPA proposed modifications to the WET test methods (66 FR 49794). The proposal included updates to the methods, minor corrections and clarifications, and specific technical changes in response to stakeholder concerns. Specifically, EPA proposed technical changes to 1) require "blocking" by known parentage in the *Ceriodaphnia dubia*

Survival and Reproduction Test; 2) specify procedures to control pH drift that may occur during testing; 3) incorporate review procedures for the evaluation of concentration-response relationships; 4) clarify recommendations regarding nominal error rate assumptions; 5) clarify limitations in the generation of confidence intervals; 6) add guidance on dilution series selection; 7) clarify requirements regarding acceptable dilution waters; and 8) add procedures for determining and minimizing the adverse impact of pathogens in the Fathead Minnow Survival and Growth Test.

EPA also solicited comment on other modifications to improve the performance of the methods, including the incorporation of variability criteria and increases in the minimum number of test replicates. EPA proposed to incorporate WET method changes into new editions of each of the WET method manuals (USEPA, 1993; USEPA, 1994a; USEPA, 1994b) and to update Table IA at 40 CFR Part 136 to cite the new method manual editions.

In the September 28, 2001 proposed rule, EPA also proposed to ratify 11 of the 12 WET methods evaluated in EPA's WET Interlaboratory Variability Study. EPA proposed to ratify the *Ceriodaphnia dubia* Acute Test; Fathead Minnow Acute Test; Sheepshead Minnow Acute Test; Inland Silverside Acute Test; *Ceriodaphnia dubia* Survival and Reproduction Test; Fathead Minnow Larval Survival and Growth Test; *Selenastrum capricornutum* Growth Test; Sheepshead Minnow Larval Survival and Growth Test; Inland Silverside Larval Survival and Growth Test; *Mysidopsis bahia* Survival, Growth, and Fecundity Test; and *Champia parvula* Reproduction Test. To support ratification of these methods, EPA presented the results of the WET Interlaboratory Variability Study (USEPA, 2001a; USEPA, 2001b), a national study of 12 WET methods involving 56 laboratories and over 700 samples. EPA proposed to withdraw *Holmesimysis costata* as an acceptable substitute species for use in the *Mysidopsis bahia* Acute

Test method protocol. In its place, EPA proposed a new *Holmesimysis costata* Acute Test protocol.

EPA invited public comment for 60 days and later extended the comment period for an additional 45 days (66 FR 58693; November 23, 2001). EPA received 38 comment packages during the allotted comment period.

#### **IV. Summary of Final Rule**

##### *A. Proposed WET Method Changes*

Today's action incorporates most of the method changes proposed on September 28, 2001 (66 FR 49794) with minor modifications to address public comments. For a summary of major changes from the proposed rule, including proposed actions not incorporated in today's rule, see Section V of this preamble. Method manual revisions promulgated in today's action include:

- Minor corrections and clarifications,
- Incorporation of updated method precision data,
- Requirement for “blocking” by known parentage in the *Ceriodaphnia dubia* Survival and Reproduction Test,
- Specification of procedures to control pH drift that may occur during testing,
- Review procedures for the evaluation of concentration-response relationships,
- Clarification of limitations in the generation of confidence intervals,
- Guidance on dilution series selection,
- Clarification of requirements regarding acceptable dilution waters,
- Procedures for determining and minimizing the adverse impact of pathogens in the Fathead Minnow Survival and Growth Test,

- Requirement for the use of ethylenediaminetetraacetic acid (EDTA) in the *Selenastrum capricornutum* Growth Test.

#### *B. Additional Revisions to WET Test Methods*

In addition to requesting comment on the specific modifications to WET test methods mentioned above, EPA solicited comment on any additional modifications that would improve the overall performance of the methods. Specifically, EPA solicited comment on application of variability criteria to test results, modification of test acceptability criteria, and increases in test replication requirements. In response to comments, today's final rule also incorporates the following additional modifications to WET test methods:

- Requirement to meet specific variability criteria when NPDES permits require sublethal WET testing endpoints expressed using hypothesis testing,
- Increases in the required minimum number of replicates for several tests,
- Clarification of required and recommended test conditions for the purposes of reviewing WET test data submitted under NPDES permits,
- Additional clarification of sample holding times,
- Clarification of requirements for reference toxicant testing and additional guidance on evaluating reference toxicant test results,
- Clarification of allowable sample holding temperatures,
- Clarification of biomass as the measured endpoint in survival and growth tests,
- Clarification of requirements for measuring total residual chlorine in WET samples,
- Modification of the test termination criteria for the *Ceriodaphnia dubia* Survival and Reproduction Test to exclude the counting of fourth brood neonates,
- Additional minor corrections identified by commenters.

### C. Ratification and Withdrawal of Methods

Based on the WET Interlaboratory Variability Study, peer review comments, and comments on the proposed rule, EPA is ratifying ten methods evaluated in the WET Interlaboratory Variability Study and withdrawing two methods. EPA is ratifying the *Ceriodaphnia dubia* Acute Test; Fathead Minnow Acute Test; Sheepshead Minnow Acute Test; Inland Silverside Acute Test; *Ceriodaphnia dubia* Survival and Reproduction Test; Fathead Minnow Larval Survival and Growth Test; *Selenastrum capricornutum* Growth Test; Sheepshead Minnow Larval Survival and Growth Test; Inland Silverside Larval Survival and Growth Test; and *Mysidopsis bahia* Survival, Growth, and Fecundity Test. In accordance with EPA's Report to Congress on the Availability, Adequacy, and Comparability of testing procedures (USEPA, 1988), EPA has confirmed that the methods ratified today are repeatable and reproducible (i.e., exhibit adequate within-laboratory and between-laboratory precision), available and applicable (i.e., adaptable to a wide variety of laboratories and use widely available organisms and supplies), and representative (i.e., predictive of receiving system impacts). See Section VI.C.1 of this preamble.

EPA's WET Interlaboratory Variability Study demonstrated that the methods ratified today generally have a high rate of successful completion, do not often produce false positive results, and exhibit precision comparable to chemical methods approved at 40 CFR Part 136. Table 1 summarizes the performance characteristics for the ten WET test methods ratified today. In ratifying these WET test methods, EPA reaffirms the conclusion expressed in the 1995 WET final rule (60 FR 53529; October 16, 1995), that these methods, including the modifications in today's rule, are applicable for use in NPDES permits.

**Table 1. Summary of performance characteristics for ratified WET methods.**

Test method	Successful test completion rate (%)	False positive rate <sup>a</sup> (%)	Interlaboratory precision (%CV) <sup>b</sup>
<i>Ceriodaphnia dubia</i> Acute Test	95.2	0.00	29.0
<i>Ceriodaphnia dubia</i> Survival and Reproduction Test	82.0	3.70	35.0
Fathead Minnow Acute Test	100	0.00	20.0
Fathead Minnow Larval Survival and Growth Test	98.0	4.35	20.9
<i>Selenastrum capricornutum</i> Growth Test	63.6	0.00	34.3
<i>Mysidopsis bahia</i> Survival, Growth, and Fecundity Test	97.7	0.00	41.3
Sheepshead Minnow Acute Test	100	0.00	26.0
Sheepshead Minnow Larval Survival and Growth Test	100	0.00	10.5
Inland Silverside Acute Test	94.4	0.00	38.5
Inland Silverside Larval Survival and Growth Test	100	0.00	43.8

<sup>a</sup> False positive rates reported for each method represent the higher of false positive rates observed for hypothesis testing or point estimate endpoints.

<sup>b</sup> Coefficients of variation (CVs) reported for each method represent the CV of LC50 values for acute test methods and IC25 values for chronic test methods. CVs reported are based on total interlaboratory variability (including within-laboratory and between-laboratory components of variability) and averaged across sample types.

EPA is withdrawing the *Holmesimysis costata* Acute Test and the *Champia parvula* Reproduction Test methods from 40 CFR Part 136. EPA was unable to obtain interlaboratory precision data for these methods in the WET Interlaboratory Variability Study due to laboratory unavailability. EPA was unable to contract with a minimum of six laboratories qualified and willing to conduct these test methods within the time frame of the Study. Due to this lack of interlaboratory precision data generated from the Study for these methods, several commenters recommended that these methods not be approved at 40 CFR Part 136 for national use. In response, today's action removes the *Holmesimysis costata* Acute Test method (1995 version) and the *Champia parvula* Reproduction Test method from the list of test methods approved for nationwide use at 40 CFR Part 136.

By withdrawing these methods from 40 CFR Part 136 for nationwide use, EPA does not reject their use on more limited bases. Today's withdrawal simply reflects that the Agency has not validated these methods for national use. EPA continues to support the use of these methods for applications other than for the determination of compliance with NPDES permit limits, as well for limited, localized, or regional use where the methods have been validated by other entities. In addition, EPA continues to support the use of the *Holmesimysis costata* Acute Test to measure toxicity to marine organisms of the Pacific Ocean. Because test procedures for measuring toxicity to estuarine and marine organisms of the Pacific Ocean are not listed at 40 CFR Part 136, permit writers may include (under 40 CFR 122.41(j)(4) and 122.44(i)(1)(iv)) requirements for the use of test procedures that are not approved at Part 136, such as the *Holmesimysis costata* Acute Test and other West Coast WET methods (USEPA, 1995b) on a permit-by-permit basis.

*D. Amendment to 40 CFR 136.3 Table IA*

Today's rule amends 40 CFR 136.3 by removing the *Champia parvula* Reproduction Test method (Method 1009.0) from Table IA, modifying the reference to acute "mysid" tests in Table IA to include only *Mysidopsis bahia* (and not *Holmesimysis costata*), adding method numbers to acute tests, revising the parameter measured in marine tests to refer to organisms "of the Atlantic Ocean and Gulf of Mexico," and modifying footnotes and references to cite the updated versions of the method manuals.



## **V. Changes from the Proposed Rule**

### *A. Proposed WET Method Changes*

On September 28, 2001, EPA proposed technical method changes to improve the performance and clarity of WET test methods and to address specific stakeholder concerns. These provisions were presented and discussed in Section III of the proposed rule preamble (66 FR 49794) and detailed in the document titled, *Proposed Changes to Whole Effluent Toxicity Method Manuals* (USEPA, 2001e). In today's action, EPA is withdrawing or revising some of the proposed revisions based on comments received on the proposed rule. These revisions are discussed below. Other comments that EPA addressed but did not result in changes from the proposal are discussed in Section VI.

#### **1. Blocking by Known Parentage**

EPA proposed specific method manual modifications that would require blocking by known parentage in the *Ceriodaphnia dubia* Survival and Reproduction Test method. Today, EPA is finalizing the proposed method changes with a minor modification to clarify that neonates from a single known parent may be used in the initiation of more than one test. This minor modification mitigates some commenters' concerns regarding the increased cost of blocking by known parentage. Blocking by known parentage requires the use of at least six neonates from each of at least ten separate parents. If more than six neonates from a given parent remain after allocating organisms to a test, those remaining neonates may be discarded, used as future culture organisms, or used in another test initiated on the same day (provided that the neonates meet age requirements).

## 2. pH Drift

During the conduct of static or static-renewal WET tests, the pH in test containers may fluctuate or drift from the initial pH value. EPA proposed specific procedures that may be used to control this pH drift in chronic WET tests. Today, EPA is revising the specified procedures in response to stakeholder comments. Some commenters requested that EPA clarify the pH that should be maintained in pH-controlled tests. Today's action clarifies that, when the test objective is to determine the toxicity of an effluent in the receiving water, the target pH to maintain in a pH-controlled test is the pH of the receiving water measured at the edge of any mixing zone authorized in a permit. When the test objective is to determine the absolute toxicity of the effluent, the target pH to maintain in a pH-controlled test is the pH of the sample upon completion of collection. The revisions also clarify that in pH-controlled tests, the pH should be maintained within  $\pm 0.2$  pH units of the target pH in freshwater chronic tests and within  $\pm 0.3$  pH units for marine/estuarine chronic tests. EPA also added guidance on interpreting the results of parallel testing.

The revisions also remove language from the proposed method manual changes that warned about effects from pH drift in the absence of pH-dependent toxicants. To address the concern that the daily cycle of pH drift and renewal caused artifactual toxicity by "shocking" test organisms, EPA proposed language in the method manuals that warned of such potential interference from pH drift even when pH-dependent toxicants were not present. EPA specifically requested that commenters provide "any data that show the value of proposed pH control measures in situations where ammonia or other pH-dependent toxicants are not present." EPA did not receive such data. EPA believes that pH drift alone is not a test interference if pH is within the organism's tolerance range. The degree of pH drift typically observed in effluent samples should generally only

interfere with test results if the sample contains a compound with toxicity that is pH dependent and at a concentration that is near the toxicity threshold. Because EPA did not receive data to suggest otherwise, EPA is removing any reference to pH drift interference in the absence of pH-dependent toxicants.

Many commenters recommended that EPA include the proposed pH control guidance for acute test methods as well as chronic methods because of the insufficiency of static renewal testing to control the pH drift and the impracticability and cost of flowthrough testing. In today's action, EPA has not provided additional techniques that involve modification of the sample to control pH drift in acute test methods, because EPA believes that the current acute methods provide adequate remedies for pH drift without modifying the sample. In acute tests, pH drift may be remedied by more frequent test renewals or use of flowthrough testing. While EPA agrees that flowthrough testing is more costly than static or static renewal testing, today's action does not impose any additional costs by requiring flowthrough testing. Today's action simply retains the options for pH control that are currently described in the acute method manual and does not add additional options.

### 3. Nominal Error Rates

Today's action does not incorporate the proposed method manual changes regarding nominal error rates. The method manuals maintain the original statement recommending a nominal error rate of 0.05. EPA proposed changes to its recommendation regarding nominal error rate assumptions, specifically, the change from 0.05 to 0.01 under specific circumstances. EPA proposed changes to its recommended error rate assumptions based on the settlement agreement, which identified the circumstances under which EPA would change its recommendations regarding nominal error rate reductions. These specified circumstances do not necessarily

represent cases where the risk of false positive results increase, but rather situations for which the petitioners sought specific relief.

Commenters on the proposed rule commented that there was no scientific justification for reducing nominal error rate assumptions in only these circumstances and recommended reducing the nominal error rate in all circumstances. EPA agrees with the commenters that there is not a scientific justification for allowing reduced nominal error rates in these specific circumstances, but disagrees that nominal error rates should be reduced in all circumstances. Some commenters claimed that a reduced nominal error rate is needed to improve confidence in the test results. Reducing the nominal error rate, however, does not inherently improve confidence in test results. Because of the relationship between Type I and Type II statistical errors, reductions in nominal error rates improve confidence in results that identify toxicity, but reduce confidence in results that do not identify toxicity. This reduces the power of the test and the chance of identifying toxic discharges, thereby reducing environmental protection. In addition, the statistical test designs (i.e., test replication requirements) of WET methods and all supporting method validation data were based on a nominal error rate of 0.05. Because there is no scientific justification for recommending reductions in nominal error rates in the circumstances proposed and commenters did not provide such supporting rationale or data, EPA has not incorporated the proposed method manual recommendations regarding nominal error rates. The method manuals maintain the original recommendation to assume a nominal error rate of 0.05.

#### 4. Dilution Series

EPA is finalizing the proposed guidance on the selection of dilution series in WET testing. In addition to the proposed guidance, EPA has made minor modifications in response to comments to further clarify that no one particular dilution series is required. Specific dilution

series used in the WET method manuals are provided as examples and recommendations, not requirements.

## 5. Dilution Waters

EPA is finalizing the proposed guidance on the selection of dilution waters in WET testing. In addition to the proposed guidance, EPA has made minor modifications in response to comments to further clarify that no single dilution water type is required for all tests. The method manuals now clarify that receiving waters, synthetic waters, or synthetic waters adjusted to approximate receiving water characteristics may be used for dilution water, provided that the water meets the qualifications for an acceptable dilution water. EPA clarified in the method manuals that an acceptable dilution water is one which is appropriate for the objectives of the test; supports adequate performance of the test organisms with respect to survival, growth, reproduction, or other responses that may be measured in the test (i.e., consistently meets test acceptability criteria for control responses); is consistent in quality; and does not contain contaminants that could produce toxicity. EPA also provided clarification on the use of dual controls. When using dual controls, the dilution water control should be used for determining the acceptability of the test and for comparisons with the tested effluent. If test acceptability criteria (e.g., minimum survival, reproduction, or growth) are not met in the dilution water control, the test must be repeated on a newly collected sample. Comparisons between responses in the dilution water control and in the culture water control can be used to determine if the dilution water, which may be a receiving water, possesses ambient toxicity.

## 6. Pathogen Interference

In today's action, EPA finalizes the proposed guidance on controlling pathogen interference in the Fathead Minnow Larval Survival and Growth Test with several modifications to address

commenter concerns. Some commenters were concerned that the proposed guidance allowed the use of pathogen control techniques such as UV, chlorination, filtration, and antibiotics only after the recommended modified test design (fewer fish per cup) failed to control pathogen interference. Today's revisions clarify that EPA recommends pathogen control techniques that do not modify the sample, such as the modified test design technique, over ones that do. Upon approval by the regulatory authority, however, analysts also may use various sample sterilization techniques that modify the sample to control pathogen interference, provided that parallel testing of altered and unaltered samples further confirms the presence of pathogen interference and demonstrates successful pathogen control.

The manuals also now provide further explanation regarding the purpose for and required extent of pathogen source determination. Commenters were concerned that EPA was requiring permittees to generate data that was irrelevant to correcting for pathogen test interference. This is not the case. Determining whether tests are adversely affected by pathogens in the effluent or pathogens in the receiving water used for test dilution is an important first step in selecting an appropriate pathogen control technique. If the source of interfering pathogens in the test is the receiving water used as the dilution water, then pathogen interference may be controlled by simply using an alternative dilution water. If the source of interfering pathogens in the test is the effluent, then pathogen control techniques are appropriate to control the interference. To further address the comments, EPA removed mention of pathogen source identification beyond determining whether the pathogen source was the effluent or dilution water. EPA also made several minor modifications in response to comments, including an acknowledgment that pathogen control techniques may not eliminate pathogens, but should minimize the adverse influence of pathogens so that test results are not confounded by mortality due to pathogens.

## 7. EDTA in the *Selenastrum capricornutum* Growth Test

In the WET Interlaboratory Variability Study, EPA found that performance of the *Selenastrum capricornutum* Growth Test was much higher (lower interlaboratory variability and lower false positive rate) when the test was conducted with EDTA (ethylenediaminetetraacetic acid). Based on this finding, EPA proposed to recommend the use of EDTA in the *Selenastrum capricornutum* Growth Test. Several commenters expressed concern that EPA only recommended, rather than required, the use of EDTA. Commenters stated that this recommendation was not sufficient to ensure the acceptable performance of the method and encouraged EPA to require the use of EDTA. To address these comments, the *Selenastrum capricornutum* Growth Test now requires the addition of EDTA to nutrient stock solutions when conducting the *Selenastrum capricornutum* Growth Test and submitting data under NPDES permits. To address concerns that EDTA may interfere with (i.e., mask) the toxicity of metals, the method continues to caution that the addition of EDTA may cause the *Selenastrum capricornutum* Growth Test to underestimate the toxicity of metals. EPA cautions regulatory authorities to consider this possibility when selecting test methods for monitoring effluents that are suspected to contain metals. As recommended in EPA's *Technical Support Document for Water Quality-Based Toxics Control* (TSD) (USEPA, 1991), the most sensitive of at least three test species from different phyla should be used for monitoring the toxicity of effluents.

### B. Additional Revisions to WET Test Methods

#### 1. Variability Criteria

Today's action incorporates mandatory variability criteria for five chronic test methods. EPA recommends the use of point estimation techniques over hypothesis testing approaches for calculating endpoints for effluent toxicity tests under the NPDES Permitting Program. However,

to reduce the within-test variability and to increase statistical sensitivity when test endpoints are expressed using hypothesis testing rather than the preferred point estimation techniques, variability criteria must be applied as a test review step when NPDES permits require sublethal hypothesis testing endpoints (i.e., no observed effect concentration (NOEC) or lowest observed effect concentration (LOEC)) and the effluent has been determined to have no toxicity at the permitted receiving water concentration. These variability criteria must be applied for the following methods: Fathead Minnow Larval Survival and Growth Test; *Ceriodaphnia dubia* Survival and Reproduction Test; *Selenastrum capricornutum* Growth Test; *Mysidopsis bahia* Survival, Growth, and Fecundity Test; and Inland Silverside Larval Survival and Growth Test. Within-test variability, measured as the percent minimum significant difference (PMSD), must be calculated and compared to upper bounds established for test PMSDs. Under this new requirement, tests conducted under NPDES permits that fail to meet the variability criteria (i.e., PMSD upper bound) and show “no toxicity” at the permitted receiving water concentration (i.e., no significant difference from the control at the receiving water concentration or above) are considered invalid and must be repeated on a newly collected sample. Lower bounds on the PMSD are also applied, such that test concentrations shall not be considered toxic (i.e., significantly different from the control) if the relative difference from the control is less than the lower PMSD bound.

In the proposed rule, EPA solicited comment on the required use of upper and lower PMSD bounds in the calculation of NOEC and LOEC values. According to the proposed approach, any test treatment with a percentage difference from the control (i.e.,  $[\text{mean control response} - \text{mean treatment response}] / \text{mean control response} * 100$ ) that is greater than the upper PMSD bound would be considered as significantly different; and any test treatment with a percentage difference



from the control that is less than the lower PMSD bound would not be considered as significantly different.

EPA received comments on this proposed approach that expressed concern that variability criteria were used only to adjust NOEC and LOEC values and not to invalidate tests. Commenters argued that the proposed approach does not control variability unless tests failing to meet the variability criteria are invalidated. In response to these comments, EPA has modified the application of variability criteria in today's action. Rather than implementing variability criteria as a component of endpoint calculation, today's method modifications implement variability criteria (upper and lower PMSD bounds) as a test review step that is required when NPDES permits require sublethal WET testing endpoints expressed using hypothesis testing for the five test methods previously listed. Reviewed tests that fail to meet the variability criteria and do not detect toxicity at the receiving water concentration are invalid and must be repeated on a newly collected sample.

EPA received comments both for and against implementation of variability criteria as test acceptability criteria. To balance these comments, the final rule implements the variability criteria as a required test review step when NPDES permits require sublethal WET testing endpoints expressed using hypothesis testing for the five test methods previously listed. As such, the variability criteria have the potential to invalidate highly variable tests. Invalidation, however, is contingent upon other data evaluation steps. For instance, tests that exceed the variability criteria are only invalidated when the test also fails to detect toxicity at the permitted receiving water concentration. The method manuals continue to restrict use of the term "test acceptability criteria" to biological measurements in test controls (i.e., control survival, reproduction, and growth) that independently assess test acceptability. Unlike the variability

criteria instituted today, the use of “test acceptability criteria” to invalidate tests are not contingent on any other data evaluation steps. For this reason, the term “test acceptability criteria” is not applicable to the variability criteria established in today’s action.

EPA received comments that recommended alternative measures for controlling within test variability, such as limits on the coefficient of variation (CV) for the control treatment. In developing variability criteria, EPA considered other measures of test precision, including the standard deviation and coefficients of variation for treatments and control, minimum significant difference (MSD), and the mean square for error from the analysis of variance of treatment effects. EPA considers the PMSD to be the measure that is most easily understood and that is most directly applied to determination of NOEC and LOEC values. The PMSD quantifies the smallest percentage difference between the control and a treatment (effluent dilution) that could be declared as statistically significant. It thus includes exactly that variability affecting determination of the NOEC and LOEC. The CV for the control or any one treatment, or selected treatments, represents only a portion of the variability affecting the NOEC and LOEC. Some State or Regional WET programs have requirements on the CV for the control and the treatment representing the receiving water concentration (RWC). Such requirements can provide finer control over the variability influencing a single comparison between the control and the RWC treatment. The PMSD upper bound provides control over the total within-test variability and is intended specifically for multi-concentration tests in which the NOEC or LOEC are determined by using hypothesis testing. Regulatory authorities may continue to use variability control strategies adopted within their jurisdiction, but when NPDES permits require sublethal WET testing endpoints expressed using hypothesis testing, the variability criteria required by today’s action must be implemented as well. Requiring such variability criteria provides national

consistency and control of WET test precision when hypothesis testing approaches are chosen. In today's action, EPA reiterates the recommendation of the method manuals and the TSD (USEPA, 1991) by stating that for the NPDES Permit Program, point estimation techniques are preferred over hypothesis testing approaches for calculating endpoints for effluent toxicity tests.

EPA received comments that the upper and lower bounds established for PMSD variability criteria were arbitrary or unrepresentative. EPA established the proposed variability criteria as performance-based standards set at the 10<sup>th</sup> and 90<sup>th</sup> percentiles of PMSD values from EPA's evaluation of national reference toxicant test data (USEPA, 2000c). In today's action, EPA has revised the variability criteria to reflect the 10<sup>th</sup> and 90<sup>th</sup> percentiles of PMSD values based on EPA's Interlaboratory Variability Study. The use of data from this study reflects not only tests performed on reference toxicants, but tests performed on effluents, receiving waters, and non-toxic "blank" samples as well. Data from this study also is representative of qualified laboratories that routinely conduct WET testing for permittees (see Section VI.C.2 of this preamble). In method development, EPA routinely uses such data from interlaboratory validation studies to set performance-based criteria.

In September 2001, EPA proposed variability criteria for four methods. Some commenters recommended that EPA expand the variability criteria to other test methods and other test endpoints. EPA did not propose variability criteria for the *Selenastrum capricornutum* Growth Test and the Sheepshead Minnow Larval Survival and Growth Test because these methods showed lower within-test variability in EPA's evaluation of national reference toxicant test data (USEPA, 2000c). EPA's WET Interlaboratory Variability Study confirmed that the Sheepshead Minnow Larval Survival and Growth Test was less variable than the methods for which EPA proposed variability criteria, however, the *Selenastrum capricornutum* Growth Test showed

comparable within-test variability to methods for which EPA proposed variability criteria. For this reason, EPA is today requiring variability criteria for the *Selenastrum capricornutum* Growth Test in addition to the four methods for which variability criteria were proposed.

As previously stated in the method manuals (USEPA, 1993; USEPA, 1994a; USEPA, 1994b) and EPA's Technical Support Document (USEPA, 1991), EPA recommends the use of point estimation techniques over hypothesis testing approaches for calculating endpoints for effluent toxicity tests under the NPDES Permitting Program. EPA is instituting variability criteria to reduce within-test variability and to increase statistical sensitivity when test endpoints are expressed using hypothesis testing rather than the preferred point estimation techniques. For the five methods for which EPA is instituting variability criteria when test results are analyzed by hypothesis test methods, less than 90% of tests are able to detect a 25% reduction in growth or reproduction (from the control treatment) as statistically significant using the hypothesis test. A 25% reduction in growth or reproduction is equivalent to the effect level measured using the preferred point estimation endpoint for chronic methods (i.e., the IC25). Instituting variability criteria for these five chronic methods will improve the overall statistical sensitivity when using hypothesis testing and allow hypothesis testing approaches to achieve a level of statistical sensitivity that is more comparable to the preferred point estimation endpoint (IC25).

EPA is not requiring variability criteria for the Sheepshead Minnow Larval Survival and Growth Test, because the WET Interlaboratory Variability Study confirmed that this method is less variable than the five methods for which EPA is requiring variability criteria. In EPA's WET Interlaboratory Variability Study, all Sheepshead Minnow Larval Survival and Growth Tests were able to detect effects of 25% or less as statistically significant in hypothesis testing without instituting variability criteria. The 90<sup>th</sup> percentile PMSD for the Sheepshead Minnow Larval

Survival and Growth Test was 17%, compared to 29%, 47%, 30%, 37%, and 28% for the five methods for which EPA is requiring variability criteria. For the chronic methods that were not evaluated in the WET Interlaboratory Variability Study, EPA does not have sufficient data to support the implementation of mandatory variability criteria at this time.

EPA is not requiring variability criteria for survival endpoints of acute methods because, in general, these methods are less variable than sublethal chronic test methods, and hypothesis testing approaches are able to achieve a level of statistical sensitivity similar to the preferred point estimation endpoint for acute methods and survival endpoints (i.e., the LC50). The preferred point estimation endpoint for the analysis of survival in acute methods is the LC50, which represents an effect level of 50% mortality. Over 90% of acute tests in the WET Interlaboratory Variability Study were able to detect effects of 50% mortality or less as statistically significant in hypothesis testing without instituting variability criteria. The 90<sup>th</sup> percentile of PMSD values in the WET Interlaboratory Variability Study was 39% for the Fathead Minnow Acute Test, 25% for the *Ceriodaphnia dubia* Acute Test, 17% for the Sheepshead Minnow Acute Test, and 31% for the Inland Silverside Acute Test. Based on these measured PMSD values, well over 90% of acute tests should be able to detect effects at the LC50 as statistically significant without instituting variability criteria.

By requiring application of variability criteria today in five methods, EPA does not intend to discourage permitting authorities from applying variability criteria for other endpoints or methods, or from applying more stringent variability criteria for the five chronic methods subject to today's action. While EPA continues to recommend that permitting authorities apply variability criteria to additional methods as recommended in EPA guidance (USEPA, 2000c), today's rule does not require such variability criteria for additional methods or endpoints.

## 2. Minimum Number of Replicates

EPA solicited comment on increasing the minimum number of replicates in certain WET tests from three to four. Commenters were supportive of this proposed change and stated that this change was needed to support the use of non-parametric hypothesis tests as outlined in the method manuals. In today's action, EPA is increasing the minimum number of replicates as proposed.

## 3. Test Requirements/Recommendations

Several commenters on the proposed rule expressed concern that WET methods do not adequately differentiate between mandatory test conditions (i.e., those required using the words “must” or “shall”) and discretionary test conditions (i.e., those recommended using the word “should”). Commenters claimed that this situation causes difficulty in reviewing, validating, and certifying test results submitted under NPDES permits. To address this concern, EPA modified the WET methods to clearly distinguish between required and recommended test conditions for the purposes of reviewing WET test data submitted under NPDES permits. In today's action, EPA has modified the tables of test conditions and test acceptability criteria presented in the method manuals for each method, such that each test condition is identified as required or recommended. In addition, EPA has added to each method manual a section on test review. This section provides guidance on the review of sampling and handling procedures, test acceptability criteria, test conditions, statistical methods, concentration-response relationships, reference toxicant testing, and test variability. This section also establishes two new requirements for WET test review: mandatory review of concentration-response relationships and, for some methods, the mandatory variability criteria described earlier.

#### 4. Sample Collection and Holding Times

In today's action, EPA has further clarified the requirements for sample collection and sample holding times. EPA made these modifications in response to comments requesting additional clarification and additional flexibility. In today's action, EPA has not modified the default maximum 36 hour sample holding time (up to 72 hours with regulatory authority approval), which must be met for first use of the sample, but EPA has provided additional clarification and additional flexibility for the use of samples for test renewals when the samples meet the initial sample holding times for first use. Sample holding times apply to "first use of the sample," and samples may be used for renewal at 24, 48, and/or 72 hours after first use.

The method manuals also now provide additional flexibility when shipment of renewal samples is delayed during an ongoing test. If shipping problems (e.g., unsuccessful Saturday delivery) are encountered with renewal samples after a test has been initiated, the permitting authority may allow the continued use of the most recently used sample for test renewal. EPA also clarified that sample collection on days one, three, and five is the recommended (not required) sample collection scheme. A minimum of three samples are required for seven-day chronic tests, but variations in the sampling scheme (i.e., the days on which new samples are collected) also are allowed.

#### 5. Reference Toxicant Testing

Today's action clarifies the purpose and requirements of reference toxicant testing and the appropriate use of reference toxicant test results. Several commenters identified inconsistencies in the requirements for reference toxicant testing and recommended that EPA clarify the purpose of generating reference toxicant test data. In today's action, EPA clarifies that reference toxicant testing is used to 1) initially demonstrate acceptable laboratory performance, 2) assess the

sensitivity and health of test organisms, and 3) document ongoing laboratory performance. EPA has made method manual modifications consistent with this stated purpose. Regardless of the source of test organisms (in-house cultures or purchased from external suppliers), the testing laboratory must perform at least one acceptable reference toxicant test per month for each type of toxicity test method conducted in that month. If a test method is conducted only monthly, or less frequently, a reference toxicant test must be performed concurrently with each effluent toxicity test. This requirement will document ongoing laboratory performance and assess organism sensitivity and consistency when organisms are cultured in-house. When organisms are obtained from external suppliers, concurrent reference toxicant tests must be performed with each effluent sample, unless the test organism supplier provides control chart data from at least the last five months of reference toxicant testing. This requirement assesses organism sensitivity and health when organisms are obtained from external vendors. To initially demonstrate acceptable laboratory performance, the method manuals require a laboratory to obtain consistent, precise results with reference toxicants before it performs toxicity tests with effluents under NPDES permits.

In today's action, EPA also clarifies the appropriate use of reference toxicant test results. Commenters recommended that EPA provide additional guidance on evaluating reference toxicant test results and using these results to validate toxicity tests on test samples of unknown toxicity. In response, EPA clarifies that reference toxicant test results should not be used as a *de facto* criterion for rejection of individual effluent or receiving water tests. Reference toxicant testing is used for evaluating the sensitivity and consistency of organisms over time and for documenting initial and ongoing laboratory performance. EPA clarified the steps to take when more than 1 in 20 reference toxicant tests falls outside of control chart limits, or when a reference



toxicant test result falls “well” outside of control limits. Under these circumstances, the laboratory should investigate sources of variability, take corrective actions to reduce identified sources of variability, and perform an additional reference toxicant test during the same month.

In response to comments that reference toxicant testing only compares variability within a laboratory, EPA added guidance for evaluating test precision among laboratories and for limiting excessive variability in reference toxicant testing. EPA has recommended that laboratories compare the calculated coefficient of variation, also referred to as the CV (i.e., standard deviation / mean), of the IC25 or LC50 for the 20 most recent data points to the distribution of laboratory CVs reported nationally for reference toxicant testing (USEPA, 2000c). If the calculated CV exceeds the 75<sup>th</sup> percentile of CVs reported nationally for LC50s or IC25s, the laboratory should use the 75<sup>th</sup> and 90<sup>th</sup> percentiles to calculate warning and control limits, respectively, and the laboratory should investigate options for reducing variability.

Several commenters recommended standardizing reference toxicants and acceptance ranges for reference toxicant test results. Other comments opposed mandatory reference toxicants and required acceptance ranges claiming that insufficient guidance and data are available for instituting such requirements and that such requirements would impose additional costs on laboratories. In today’s action, EPA is not requiring the use of specific reference toxicants or setting required acceptance ranges for reference toxicant testing. EPA agrees that requiring specific reference toxicants and acceptance ranges would increase laboratory costs. Many laboratories would be forced to develop initial and ongoing documentation of laboratory performance (e.g., reference toxicant control charts) using a new reference toxicant. For these laboratories, years of historic performance information using the original reference toxicant would be rendered useless. In addition, EPA believes that certain advantages gained by requiring

reference toxicant acceptance ranges are already provided by method modifications instituted in today's action. For instance, today's action institutes variability criteria when NPDES permits require sublethal WET testing endpoints expressed using hypothesis testing. This method modification limits WET test variability, which would be one of the primary purposes of any standardized reference toxicant acceptance ranges.

#### 6. Sample Holding Temperature

Today's action clarifies the allowable sample holding temperatures for WET samples as 0/-6/C. EPA received comments that the Agency should establish acceptable ranges for the current sampling holding temperature of 4/C. EPA has defined the acceptable range as 0/-6/C based on current NELAC (National Environmental Laboratory Accreditation Conference) standards which state that, "for samples with a specified storage temperature of 4/C, storage at a temperature above the freezing point of water to 6/C shall be acceptable" (NELAC, 2001). EPA also clarifies that hand-delivered samples used on the day of collection do not need to be cooled to 0/-6/C prior to test initiation.

#### 7. Biomass

Today's action clarifies that the sublethal endpoint used in survival and growth tests is based on the number of initial organisms exposed. Comments expressed concern that by calculating the chronic endpoint based on the number of initial organisms (rather than surviving organisms), the growth endpoint was in error and biased. EPA disagrees. In the 1995 WET final rule, EPA changed the test endpoint from a growth endpoint that was based on the number of surviving organisms, to a combined growth and survival endpoint that is based on the number of initial organisms. This does not represent an error in the endpoint calculation, but rather a change in the endpoint itself. EPA made this change: 1) to provide consistency with other methods (e.g.,

*Ceriodaphnia dubia* Survival and Reproduction Test) that incorporate survival along with sublethal effects, and 2) because the survival and growth endpoint is a more sensitive measure than the growth endpoint alone. While the 1995 WET final rule changed the test endpoint to a combined survival and growth endpoint, the method manuals continued to refer to the endpoint as a “growth” endpoint. Today’s action clarifies that the endpoint is, in fact, a combined survival and growth endpoint that is more accurately termed biomass.

#### 8. Total Residual Chlorine

Today’s action clarifies the requirements for measuring total residual chlorine in WET test samples. Several commenters stated that certain requirements for measuring total residual chlorine were unnecessary when the absence of the chemical has already been determined. In response to these comments, EPA has clarified that if total residual chlorine is not detected in effluent or dilution water at test initiation, it is unnecessary to measure total residual chlorine at test solution renewal or at test termination. If total residual chlorine is detected at test initiation, then measurement of total residual chlorine at test solution renewal and test termination would continue to be required. EPA also has clarified that the measurement of total residual chlorine is unnecessary in laboratory prepared synthetic dilution water.

Commenters also recommended that EPA remove the requirement for the analysis of total residual chlorine immediately following sample collection. EPA has maintained this requirement in today’s action, because information on chlorine at the site and time of collection is important for evaluating the effectiveness of chlorination/dechlorination processes and comparing the results of WET testing with instream effects.

## 9. *Ceriodaphnia dubia* Survival and Reproduction Test Termination Criteria

Commenters recommended various modifications to the test termination criteria in the *Ceriodaphnia dubia* Survival and Reproduction Test. Some commenters recommended a strict seven-day test, and others recommended that the test last no longer than seven days. Other commenters recommended that the test be terminated when 80% of control females produce three broods, rather than the current criteria of 60%. Still other commenters recommended that fourth brood neonates not be counted. To evaluate the recommended approaches to terminating *Ceriodaphnia dubia* Survival and Reproduction Tests, EPA analyzed test data from the WET Interlaboratory Variability Study using each of the recommended test termination criteria. EPA compared the recommended criteria to the current criteria by calculating within-test variability and successful test completion rates under each of the test termination scenarios. While some of the recommended test termination criteria (such as termination when 80% of control females produce three broods or a maximum of seven days) slightly improved the within-test variability of the method (from a median PMSD of 23.2% to 19.9%), these criteria caused significant reductions in successful test completion (from 83% successful completion to 66%). Only the recommendation to exclude fourth brood neonates resulted in a decrease in within-test variability without an offsetting decrease in the rate of successful test completion. Based on these results, EPA is modifying the *Ceriodaphnia dubia* Survival and Reproduction Test to specify that neonates from fourth broods are excluded from the number of neonates counted in the test. With the exception of excluding fourth brood neonates, EPA is maintaining the current test termination criteria. These criteria state that the test is terminated when 60% or more of the surviving control females have produced their third brood, or at the end of eight days, whichever occurs first. These criteria may be met at six, seven, or eight days.

## 10. Additional Minor Corrections

Some commenters identified additional errors in the WET method manuals or the proposed changes that EPA was not aware of at the time of proposal. In today's action, EPA has made these additional corrections and minor clarifications.

### *C. Ratification and Withdrawal of Methods*

In the September 28, 2001 proposal, EPA proposed to ratify the following eleven test methods evaluated in the WET Interlaboratory Variability Study: *Ceriodaphnia dubia* Acute Test; Fathead Minnow Acute Test; Sheepshead Minnow Acute Test; Inland Silverside Acute Test; *Ceriodaphnia dubia* Survival and Reproduction Test; Fathead Minnow Larval Survival and Growth Test; *Selenastrum capricornutum* Growth Test; Sheepshead Minnow Larval Survival and Growth Test; Inland Silverside Larval Survival and Growth Test; *Mysidopsis bahia* Survival, Growth, and Fecundity Test; and *Champia parvula* Reproduction Test. EPA proposed to withdraw the *Holmesimysis costata* Acute Test and, in its place, proposed a revised version of the method. As explained previously, EPA is ratifying ten of these methods today based on the results of EPA's WET Interlaboratory Variability Study that demonstrate the adequacy, availability, and comparability of the methods (see Section IV.C). For these ten methods, EPA generated sufficient interlaboratory validation data, and those data justify ratification. EPA's WET Interlaboratory Study evaluated interlaboratory precision, successful test completion rates, and false positive rates of the WET methods from the testing of over 700 samples in 56 laboratories. For each method ratified in today's action, EPA obtained interlaboratory data on four sample matrices from at least seven laboratories to as many as 35 laboratories.

Several commenters expressed concern that EPA did not properly validate WET test methods, specifically, the *Champia parvula* Reproduction Test and the *Holmesimysis costata*

Acute Test. EPA was unable to obtain interlaboratory precision data for these methods in the WET Interlaboratory Variability Study. Because these WET methods are not used widely in NPDES permits, EPA was unable to contract with a minimum of six laboratories qualified and willing to conduct these test methods within the time frame of the Study. In the proposed rule, EPA supported these methods with intralaboratory precision data and limited interlaboratory precision data (two trials of the *Holmesimysis costata* Acute Test in two laboratories), but commenters questioned the sufficiency of such data for validating methods for nationwide use, as well as the necessity to approve such methods for nationwide use.

EPA has reviewed its proposal to ratify the *Champia parvula* Reproduction Test in light of comments received and has decided to withdraw the method from the list of nationally-approved test methods at 40 CFR Part 136. At the current time, an insufficient number of laboratories nationwide have the capabilities to perform the method. As noted, EPA was thus unable to obtain a rigorous multi-laboratory performance data set to comprehensively evaluate this method. EPA had predicted that as the requirements for use of this organism in the NPDES permit program increased, the resulting increase in market demand would result in an increase in the number of laboratories capable of performing the test. However, the number of permits requiring the *Champia parvula* chronic test has remained low (DeGraeve et al., 1998), so few laboratories have invested in developing *Champia parvula* cultures or standard operating procedures for the method. While today's action removes the *Champia parvula* chronic test method from the 40 CFR Part 136 listing, EPA retains the standardized method in the marine chronic method manual with an explanation that the method is not listed at 40 CFR Part 136 for nationwide use. Accordingly, retention of the method in the method manual continues to enable standardization of

the method for developmental and other non-regulatory purposes and may foster laboratories to maintain or even develop expertise in performing the method.

EPA also has reviewed its proposal of the *Holmesimysis costata* Acute Test in light of comments received. As proposed, EPA now withdraws *Holmesimysis costata* as an acceptable species for use in the *Mysidopsis bahia* Acute Test method. EPA does not, however, promulgate the proposed *Holmesimysis costata* Acute Test method as a nationally-approved method at 40 CFR Part 136 at this time. Because the *Holmesimysis costata* Acute Test is used in only a small number of permits on the West Coast, EPA was unable to obtain sufficient interlaboratory data on this method during the time that the WET Interlaboratory Variability Study was conducted to support today's rulemaking. While today's action removes the *Homesimysis costata* Acute Test from the 40 CFR Part 136 listing, EPA includes the proposed method in the method manual with an explanation that the method has not yet been approved at 40 CFR Part 136 for nationwide use.

Three commenters, including the California State Water Resources Control Board, supported ratification of the *Holmesimysis costata* Acute Test method. The California State Water Resources Control Board added that ratification of this method was "particularly important, as it is the only method employing a marine species that is indigenous to the Pacific coast." The California State Water Resources Control Board has been proactive in developing, testing, validating, and implementing WET test methods specific to West Coast species (USEPA, 1995b), and EPA does not intend to frustrate that effort by today's action. For this reason, EPA is specifying in Table IA of 40 CFR Part 136 that the marine acute and marine chronic test methods ratified in today's rulemaking measure toxicity to estuarine and marine organisms "of the Atlantic Ocean and Gulf of Mexico." By defining the parameter measured by promulgated marine methods as toxicity to organisms "of the Atlantic Ocean and Gulf of Mexico," today's action does

not displace West Coast methods that have been approved for use in States such as California. Because test procedures for measuring toxicity to estuarine and marine organisms of the Pacific Ocean are not listed at 40 CFR Part 136, permit writers may include (under 40 CFR 122.41(j)(4) and 122.44(i)(1)(iv)) requirements for the use of test procedures that are not approved at Part 136, such as West Coast WET methods (USEPA, 1995b) on a permit-by-permit basis. Furthermore, this rule does not preclude permit writers addressing marine or estuarine waters of the Pacific Ocean from requiring, on a permit-by-permit basis, any method designated as approved for "estuarine and marine organisms of the Atlantic Ocean and Gulf of Mexico," where such method is suitable for the specific application.

## **VI. Response to Major Comments**

EPA encouraged public participation in this rulemaking and requested comments on the proposed revision and ratification of WET methods. EPA also requested data supporting comments, if available. Thirty-eight stakeholders provided comments on the proposal. Stakeholders included eight laboratories, eight regulatory authorities, 11 industries/industry groups, nine publicly-owned treatment works (POTWs), and two environmental consulting companies.

This section summarizes major comments received on the proposed rule that were not previously addressed in Section V and provides a summary of EPA's responses. The complete comment summary and response document can be found in the public record for this final rule.

### *A. Proposed WET Method Changes*

EPA received comments on each of the proposed method changes, and those comments that prompted modifications to the proposed method changes are discussed in Section V of this preamble. Other substantial comments on proposed method changes follow.



## 1. Cost

Several commenters expressed concern that proposed method modifications will increase test costs. Of the WET method modifications instituted in today's action, only four are additional mandatory changes that have the potential to increase test costs. These four modifications include: 1) the requirement for blocking by known parentage in the *Ceriodaphnia dubia* Survival and Reproduction Test; 2) the requirement to review test results for concentration-response relationships; 3) the incorporation of mandatory variability criteria for certain test methods when NPDES permits require sublethal WET testing endpoints expressed using hypothesis testing; and 4) the increase in the minimum number of replicates for the Fathead Minnow Larval Survival and Growth Test, *Selenastrum capricornutum* Growth Test, Sheepshead Minnow Larval Survival and Growth Test, Inland Silverside Larval Survival and Growth Test, and Sea Urchin Fertilization Test. EPA believes that the overall cost increases due to these changes will be minor and that the potential benefits of these modifications outweigh the incremental costs. EPA has estimated that the total cost of these modifications for all permittees will be less than five million dollars per year nationwide for all tests (Table 2 and USEPA, 2002). EPA believes that these costs also would be alleviated by a potential reduction in costs for retesting and additional investigations (e.g., toxicity identification evaluations). The modifications should result in improved test performance and increased confidence in the reliability of testing results.

**Table 2. Estimated total cost resulting from WET method modifications required by today's action (from USEPA, 2002).**

<b>Modification</b>	<b>Cost (\$/yr)</b>
Blocking-by-parentage	\$352,592
Concentration-response relationship	\$98,069
Increased replicates	\$886,634
Variability criteria	\$2,595,873
<b>Total</b>	<b>\$3,933,168</b>

## 2. Concentration-Response Relationships

Today, EPA is finalizing proposed method modifications to require the review of concentration-response relationships for all multi-concentration tests. Under this requirement, the concentration-response relationship generated for each multi-concentration test must be reviewed to ensure that calculated test results are interpreted appropriately. In conjunction with this requirement, EPA has provided recommended guidance for concentration-response relationship review (USEPA, 2000a).

Several commenters expressed concern that the proposed method modifications require that the concentration-response relationship be reviewed but does not require that a concentration-response relationship be established before determining that toxicity is present. Commenters recommended that EPA require the establishment of a “valid” concentration-response relationship prior to determining toxicity. Though within the scope of the proposed rule, EPA does not consider such a requirement appropriate for several reasons. First, WET methods and the WET testing program rely on the measurement of specific test endpoints (NOECs, LC50s, IC25s) for determining toxicity, not on achievement of specified concentration-response patterns. Second,

the concentration-response guidance is a component of test review that ensures that test endpoints, which are used to determine toxicity, are calculated and interpreted appropriately. Second, concentration-response relationships are empirical; and a single definition for a “valid” concentration-response relationship is not appropriate. A range of toxicants may produce an infinite range of different shaped responses. In addition, a single response pattern may be due to several different reasons, some indicating toxicity, and some not. For example, the presence of pathogens, considered an adverse effect confounding WET tests, may produce the same concentration-response pattern as a true toxicant. For this reason, EPA designed the guidance as a step-by-step review process that investigates the causes for non-ideal concentration-response patterns and provides for proper interpretation of test endpoints. Third, WET testing has inherent characteristics that may limit the ability to achieve ideal concentration-response relationships. For instance, WET testing is constrained to 100% effluent sample as the highest test concentration. This sometimes inhibits the ability to establish an ideal concentration-response relationship that extends gradually from no effect at one concentration to complete effect at some higher concentration. Traditional toxicology on pure substances, from which the concentration-response relationship concept is borrowed, is not similarly constrained. Test concentrations can be increased or lowered until an ideal response is generated. The typical WET test design of five concentrations and a control also may limit the ability to generate ideal concentration-response relationships. The location or spacing of these five concentrations may miss the gradual transition from no effect to complete effects. In traditional toxicology using pure substances, tests can be rerun with altered or additional test concentrations of the same compound, but in WET testing each individual sample and test is unique and cannot be exactly duplicated due to the complex and dynamic nature of the test samples over time. Non-ideal concentration-response

relationships will occasionally be encountered in WET testing, and the goal of concentration-response relationship review is to properly interpret these non-ideal patterns.

Fourth, the concentration-response relationship guidance has been shown to be very effective at reducing false positives. For instance, in the WET Interlaboratory Variability Study, the use of the concentration-response relationship guidance reduced false positive incidences from above 14% to below 5% for some methods (USEPA, 2001a).

### 3. Confidence Intervals

EPA is finalizing the proposed method modifications that provide guidance when confidence intervals are not generated. This guidance clarifies that confidence intervals may not be generated by EPA software when test data do not meet specific assumptions required by the statistical methods, when point estimates are outside of the test concentration range, or when specific limitations imposed by the software are encountered. EPA also provides guidance for proceeding under each circumstance. Some commenters stressed the importance of obtaining confidence intervals in all circumstances and recommended that EPA use confidence intervals in assessing the reliability of results and determining compliance. EPA believes that the failure to generate confidence intervals should not adversely affect WET test result reporting because confidence intervals surrounding point estimates are not currently reported in the Permit Compliance System (the national database tracking compliance with NPDES permits) or used in compliance determinations. Compliance with permit requirements is based on the point estimate itself and not confidence intervals surrounding the estimate. This approach is no different in WET testing than in chemical testing, where compliance is also based on the analytical result itself. EPA demonstrated in the WET Interlaboratory Variability Study that the WET methods

provide adequate precision and adequate protection from false positives. Therefore, EPA is not altering the compliance determination approach to include the use of confidence intervals.

#### *B. Additional Revisions to WET Test Methods*

In addition to receiving comment on proposed method modifications, EPA received comments recommending additional method modifications. Those recommendations that EPA incorporated in today's action and those comments that prompted additional modifications are discussed in Section V of this preamble. Other substantial comments on additional method changes are discussed below.

##### **1. Method Flexibility**

EPA received comments that requested additional requirements be added to WET test methods, as well as comments that WET test methods are overly restrictive and would benefit from additional flexibility. As with all promulgated methods, EPA has attempted to balance these two opposing objectives. EPA has prescribed certain method elements when necessary to ensure the reliability of results, and allowed flexibility in other method elements so that the performance of analytical methods can be optimized. As noted in Section V.B.3, EPA reevaluated the use of mandatory and discretionary terms in the WET test methods to ensure that the terms are included in the manuals as intended.

EPA received comments that WET test methods do not adequately distinguish between required and recommended procedures. In response, EPA modified the tables of test conditions and test acceptability criteria presented in the method manuals for each method, such that each item is identified as required or recommended. In addition, EPA added to each method manual a section on test review. This section provides direction on the review of sampling and handling

procedures, test acceptability criteria, test conditions, statistical methods, concentration-response relationships, reference toxicant testing, and test variability.

EPA believes that these method modifications clarify the requirements for acceptable WET test results submitted under NPDES permits. However, EPA acknowledges that these method modifications will not solve all commenters concerns regarding inconsistencies in WET test review and acceptance. In the WET test methods, EPA established the minimum requirements for acceptable WET tests. In some cases, NPDES permits incorporate recommendations from the WET test method manuals as requirements in the permit (on a permit-by-permit basis). Authorized States retain the authority to establish more stringent requirements or to require additional procedures, test conditions, or QC elements. Thus, WET requirements ultimately reflected as NPDES permit requirements may continue to differ among States.

## 2. Test Acceptability Criteria

In the proposed rule, EPA solicited comments on increasing the test acceptability criteria for mean control reproduction in the *Ceriodaphnia dubia* Survival and Reproduction Test and mean control weight in the Fathead Minnow Larval Survival and Growth Test. EPA also requested that commenters submit supporting data. EPA received comments both in favor of and opposed to increasing test acceptability criteria for these methods, but these comments were not accompanied by supporting data. Because EPA does not currently possess and did not receive data indicating that such changes would improve the performance of the methods, EPA is not modifying the survival, growth and reproduction test acceptability criteria for these methods in today's action.

EPA also received comments recommending the Agency establish requirements for additional test acceptability criteria, such as limits on control variability. Today's action does establish mandatory variability criteria when NPDES permits require sublethal WET testing

endpoints expressed using hypothesis testing. EPA has incorporated these variability criteria as a required test review step for five methods rather than as test acceptability criteria, meaning that, depending on the reviewed result, retesting may be necessary. EPA continues to use the term “test acceptability criteria” only to refer to the evaluation of biological measurements in test controls (i.e., control survival, reproduction, and growth).

### 3. Quality Assurance/Quality Control Requirements

Some commenters expressed concern that WET test methods do not contain adequate quality assurance/quality control (QA/QC) requirements. Each of the toxicity test method manuals contains separate, detailed, QA/QC guidelines, and each analytical method within these manuals discusses all aspects of the tests which are related to QA/QC. Section 4 of each method manual provides QA/QC requirements and guidance for facilities, equipment, and test chambers; test organisms; culturing and test dilution water; effluent and receiving water sampling and handling; test conditions; food quality; test acceptability criteria; calibration and standardization; replication and test sensitivity; demonstrating acceptable laboratory performance; documenting ongoing laboratory performance; and record keeping. The primary QA/QC requirements of WET test methods, as contained in Section 4 of the method manuals, remain the requirements for acceptable biological performance (survival, reproduction, and growth) in test controls and the requirement for the routine analysis of reference toxicants. In today’s action, however, EPA added additional QA/QC requirements including the required review of concentration-response relationships and mandatory variability criteria when NPDES permits require sublethal WET testing endpoints expressed using hypothesis testing. EPA believes that the QA/QC requirements of WET tests will adequately ensure that results are reliable and of known and acceptable quality.

### 4. Statistical Methods

Several commenters recommended that EPA approve and use alternative statistical methods (such as percent effect approaches and Generalized Linear Models). EPA has not included such alternative statistical methods in today's modifications to WET test methods. EPA believes that the statistical methods currently recommended in the WET methods are appropriate, and acknowledges that these recommended statistical methods are not the only appropriate techniques. The method manuals state that, "the statistical methods recommended in this manual are not the only possible methods of statistical analysis." The recommended statistical methods described in the method manuals were selected because they are "(1) applicable to most of the different toxicity test data sets for which they are recommended, (2) powerful statistical tests, (3) hopefully 'easily' understood by nonstatisticians, and (4) amenable to use without a computer, if necessary" (see Subsection 9.4.1.2 of USEPA, 1994a).

Several commenters also expressed concern over bias introduced by the smoothing technique that is used in the recommended Inhibition Concentration Procedure (ICp). EPA has acknowledged in the method manuals and in method guidance (USEPA, 2000a) that the smoothing process may result in an upward adjustment in the control mean. EPA has provided guidance on concentration-response relationship review that corrects anomalous results that may arise from this smoothing procedure (USEPA, 2000a). This guidance warns that results from point estimation techniques should be interpreted carefully when the response pattern includes stimulation at low concentrations and no significant effect at higher concentrations. Under these conditions, the smoothing process could result in anomalous results, so EPA guidance recommends evaluating the ICp calculation without smoothing in these cases. If the percent effect at the receiving water concentration (RWC) is less than 25% when calculated without smoothing, and the response at the RWC is not statistically significantly different from the



control response, then a calculated IC25 of less than the RWC should be noted as anomalous and the effluent determined to be non-toxic at the RWC.

### *C. Ratification and Withdrawal of Methods*

#### 1. Validation of Performance Characteristics

Several commenters stated that EPA did not properly validate WET test methods because it did not evaluate essential performance characteristics. Commenters referenced EPA's *Report to Congress on the Availability, Adequacy, and Comparability of Testing Procedures* (USEPA, 1988) and stated that EPA failed to validate the following performance characteristics required by this report: accuracy, precision, dynamic range, detection limits, interferences, ruggedness (applicability), reporting, and representativeness/method comparability. EPA disagrees with this assertion and maintains that the WET test methods ratified in today's action were adequately validated according to all of the applicable criteria identified in the 1988 Report to Congress.

The list of performance characteristics cited by the commenters is provided in the 1988 Report to Congress within the context of chemical methods, and several of these characteristics are not applicable to biological test methods such as the WET methods that EPA is ratifying today. The 1988 Report to Congress specifically notes that not all such criteria apply to biological testing. The Report explains that the generation of scientifically accurate and valid biological measurements for environmental pollutants requires approximately the same criteria for assessing the adequacy of a method as previously described for chemical analyses, however, there are several differences which are important. Detection limits and dynamic range are specifically listed as characteristics that "are not usually appropriate concepts for all biological measurements unless instrumentation is required." Because some performance characteristics listed in the 1988 Report to Congress for chemical methods are not applicable to biological test

methods, EPA did not (and, in fact, could not) evaluate those inapplicable performance characteristics for WET test method validation.

In ratifying the previously approved WET test methods, EPA applied the availability, adequacy, and comparability criteria identified in the Report as relevant to biological measurements. The WET test methods ratified today are “available” because EPA has identified a sufficient number of laboratories that can conduct the test and culture the test organisms. The ratified WET test methods are “adequate” because the multi-laboratory tests (as well as aggregation of single laboratory tests) demonstrate high degrees of precision; the tests are reproducible. In addition, the manuals identify interferences and ways to control interference. Finally, the test acceptability criteria for control performance and requirements for reference toxicant testing provide sufficient standards to ensure data integrity, absent the “calibration” procedures available with non-living analytical instrumentation.

The Report specifically identified detection limits and dynamic range as performance characteristics that are usually not applicable to biological measurements, and the 1988 conclusions remain true today. In addition, accuracy is a performance characteristic that is not completely applicable to WET testing. Accuracy as a performance characteristic of a measurement system describes the closeness of measured results to a known result. Chemical methods generally measure some surrogate property (e.g., absorption of light at a particular wavelength) of an analyte (e.g., copper) to determine the concentration of that analyte. To confirm that the surrogate measure accurately represents the true concentration of the analyte, the pure analyte can be weighed, diluted to a known concentration, and measured using the analytical procedure under study. This procedure cannot be conducted for whole effluent toxicity. Toxicity cannot be purified, weighed, or diluted to a known concentration of “toxicity.” Toxicity is only

defined by its effects on organisms, and it is these effects that are directly measured in the toxicity test. Because toxicity is inherently defined by the measurement system (a “method-defined analyte”), and toxicity cannot be independently measured apart from a toxicity test, accuracy as a performance characteristic is not completely applicable. The inapplicability of the accuracy performance characteristic does not mean that WET tests are not accurate or that permittees are incapable of certifying the accuracy of WET test results reported on discharge monitoring reports. It means simply that the procedures commonly used in analytical testing to measure the performance characteristic that is termed “accuracy” cannot be applied to WET test methods.

Notwithstanding the previous explanation, one component of accuracy can be described for WET tests. The American Society for Testing and Materials (ASTM) defines accuracy as “a measure of the degree of conformity of a single test result generated by a specific procedure to the assumed or accepted true value and includes both precision and bias” (ASTM, 1998; emphasis added). Bias is defined as “the persistent positive or negative deviation of the average value of a test method from the assumed or accepted true value” (ASTM, 1998). Precision is defined as “the degree of agreement of repeated measurements of the same property, expressed in terms of dispersion of test results about the arithmetical mean result obtained by repetitive testing of a homogeneous sample under specified conditions” (ASTM, 1998). Like ASTM, the 1988 Report to Congress (USEPA, 1988) also explains that accuracy includes both bias and precision. As explained previously, EPA conducted an Interlaboratory Variability Study of the ratified methods in order to, among other things, generate a quantified estimate of the precision for each method studied. WET tests are therefore amenable to the precision portion of accuracy. It is the bias portion of accuracy that is not applicable to WET test methods and cannot be described for WET as it is described for chemical analytes.

The additional performance characteristics listed in the 1988 Report to Congress, namely precision, interferences, ruggedness (applicability), reporting, and representativeness, are applicable to biological test methods, and EPA evaluated and considered these characteristics in ratifying the WET test methods. To establish the precision of the methods, EPA conducted an Interlaboratory Variability Study for each of the WET methods ratified today. From the Study, EPA established single-laboratory and multi-laboratory precision estimates for multiple sample matrices for each method (USEPA, 2001a; USEPA, 2001b). EPA also conducted a study of within laboratory precision measured when testing reference toxicants (USEPA, 2000c). In today's action, EPA is modifying the WET method manuals to include this new and updated single-laboratory and multi-laboratory precision data for each method. Precision data from the WET Interlaboratory Variability Study confirmed that the WET test methods provided adequate precision (CVs ranged from 10.5 to 43.8%). The measured precision ranges for the ratified toxicity tests demonstrate the tests are comparable to (no more variable than) chemical analytical methods approved at 40 CFR Part 136. Finally, the precision had improved since the time the methods were promulgated in 1995, thus confirming EPA's conclusions that precision would improve with time, i.e., as analysts developed more expertise the methods would be "validated by use."

In addition to precision, EPA evaluated and considered the performance characteristic of interferences. Each WET test method contains a section describing possible test interferences. In today's action, EPA has expanded that section to address two additional interference concerns that were raised by stakeholders by including guidance for controlling test interference that could be due to pH drift in the test and interference caused by pathogens.

EPA also evaluated and considered the performance characteristic of ruggedness or applicability. The methods ratified today use materials that are widely available and organisms that can be easily cultured in the laboratory. By conducting a national interlaboratory study of these methods, EPA also confirmed that the methods are adaptable to a wide variety of laboratories and that the methods generate reproducible results in those laboratories. In the WET Interlaboratory Variability Study, EPA documented successful test completion rates of 63.6% to 100% for WET methods. EPA anticipates that method modifications instituted today will improve the successful test completion rate for methods at the bottom of this range, such as the *Selenastrum capricornutum* Growth Test. Today, EPA is requiring the use of EDTA in this test. As laboratories gain experience in performing the test with EDTA, EPA anticipates that successful test completion rates will improve. See Section VI.C.4 of this preamble.

EPA also considered the aspect of result reporting in its development and validation of WET test methods. Each method manual contains a section devoted to test review and reporting. In today's action, EPA has supplemented this section by providing guidance on the review of sampling and handling, test acceptability criteria, test conditions, statistical methods, concentration-response relationships, reference toxicant testing, and test variability. In addition, EPA clarified the required and recommended test conditions when submitting data under NPDES permits.

EPA documented and considered the representativeness or comparability of WET methods. Prior to approving the WET test methods in the 1995 WET final rule, EPA conducted several studies that demonstrated the ability of WET tests to predict impacts of effluents on the biological integrity of receiving waters (USEPA, 1991). In a 1995 workshop of nationally recognized WET experts (the Society of Environmental Toxicology and Chemistry's Pellston Workshop),

including those from academia, government, and the regulated community (e.g., POTWs and industry), the experts concluded that “WET testing is an effective tool for predicting receiving system impacts when appropriate considerations of exposure are considered” (Waller et al., 1996). The workgroup also agreed that “further laboratory-to-field validation is not essential for the continued use of WET testing” (Waller et al., 1996).

## 2. Interlaboratory Variability Study

Several commenters expressed concern that EPA used data from the Interlaboratory Variability Study that was of poor quality and would have been discarded in a regulatory context. In conducting the WET Interlaboratory Variability Study, EPA’s objective was to validate the WET methods as promulgated. EPA was not attempting to validate the diversity of testing requirements that may be implemented in various States. State regulatory authorities retain the discretion to enhance the requirements of a method for implementation in their State as well as to require procedures that EPA otherwise recommends. In the WET Interlaboratory Variability Study, EPA appropriately evaluated data according to the promulgated methods and ASTM guidance for measuring interlaboratory method precision. EPA accurately invalidated tests according to test acceptability criteria specified in each method. EPA acknowledges that the promulgated methods allow flexibility in the review of test conditions. The method manuals state that departures in specified test condition ranges do not necessarily invalidate test results. In today’s action EPA modified the methods to better clarify this allowable flexibility. For the purposes of reviewing data submitted under NPDES permits, the manuals now clearly distinguish between requirements of the method and recommended test condition ranges.

Several commenters expressed concern that EPA did not use the results of reference toxicant tests from the WET Interlaboratory Variability Study to qualify or disqualify data. EPA agrees.

EPA used reference toxicant tests in the manner in which they are described in the method manuals. Failure of reference toxicant tests do not necessarily invalidate a test. In today's action, EPA has incorporated method modifications to clarify reference toxicant testing requirements and the appropriate use of reference toxicant test data. EPA has clarified that reference toxicant test results should not be used as a *de facto* criterion for rejection of individual effluent or receiving water tests, but rather, reference toxicant testing is used for evaluating the health and sensitivity of organisms over time and for documenting initial and ongoing laboratory performance.

Several commenters expressed concern that too few data points were used to estimate method performance in the WET Interlaboratory Variability Study. In accordance with ASTM guidance on determining interlaboratory method precision, EPA set a data quality objective of a minimum of six complete and useable data sets for each WET test method evaluated in the Study. To meet this data quality objective, EPA endeavored to sponsor a minimum of nine laboratories per method. For all of the methods that EPA is ratifying today, seven or more laboratories participated in interlaboratory testing. For several individual sample matrices and test method combinations that were tested (blank sample analyzed using the *Selenastrum capricornutum* Growth Test, receiving water sample analyzed using the *Selenastrum capricornutum* Growth Test without EDTA, and the receiving water sample analyzed using the Inland Silverside Acute Test), fewer than six useable data sets were obtained. EPA did not, however, establish precision criteria in today's rule based on results from a single sample matrix. EPA tested four sample matrices (blank, reference toxicant, effluent, and receiving water) with each test method, and precision estimates were based on the combined results of reference toxicant, effluent and receiving water testing. Because multiple sample matrices were used to generate precision estimates, more than

six useable data sets were used for each method. In fact, at least 17 data sets were used to establish precision estimates for each method.

Several commenters also expressed concern that the selection of laboratories for the WET Interlaboratory Variability Study was biased. EPA disagrees. EPA believes that the laboratories that participated in the WET Interlaboratory Variability Study were representative of the laboratory community that commonly conducts WET testing for permittees. From the outset, EPA and the regulated community wanted to ensure that participants in the Study were representative. Industry trade groups, such as AMSA (Association of Metropolitan Sewerage Agencies), surveyed their member permittees to identify the laboratories that provide their routine WET testing services. AMSA requested that members sponsor those laboratories' participation in the Study. Of the 55 participant laboratories involved in the Study, 44 (or 80%) were specifically recommended by AMSA with commitments from AMSA members to sponsor such laboratories' participation in the Study. Thirty-seven of these laboratories were ultimately sponsored by AMSA members to analyze samples using one or more methods. The remaining seven laboratories had commitments of sponsorship from AMSA members, but were ultimately sponsored by EPA in the Study because their bids were among the nine lowest. The high percentage (80%) of laboratories in the Study that were sponsored by permittees for participation demonstrates that the laboratories involved in the Study are representative of those that commonly conduct WET testing for permittees.

Several commenters expressed concern that a majority of laboratories did not detect toxicity in the reference toxicant sample type distributed for the *Ceriodaphnia dubia* Survival and Reproduction Test method. Prior to interlaboratory testing in the WET Interlaboratory Variability Study, referee laboratories conducted preliminary testing to determine the appropriate



composition of samples to prepare for the Study. This preliminary testing was important for ensuring that test samples prepared for the Study produced results within the test concentration range. Despite these preliminary testing efforts, the spiking level selected for the reference toxicant sample type in the *Ceriodaphnia dubia* Survival and Reproduction Test method was insufficient to produce the targeted level of effect. The spiking concentration of KCl for this sample was selected to achieve an IC25 of approximately 50% sample based on preliminary testing, but the spiked sample missed this targeted effect level. The prepared sample was only slightly toxic and could not be detected as toxic in 67% of tests. Depending on the sensitivity of test organisms at individual laboratories, some laboratories identified the sample as toxic, while other laboratories did not. Similarly, marginally toxic effluents may exhibit intermittent toxicity in routine monitoring. In such cases, permittees and regulatory authorities should consult EPA guidance that addresses marginal and intermittent toxicity (USEPA, 1991; USEPA, 2000c; USEPA, 2001f).

The reference toxicant sample used in the Study also was prepared as an ampule that was reconstituted at each participant laboratory. This reconstitution process also likely produced minor variations (from laboratory to laboratory) in the final sample composition that influenced whether toxicity was detected. While the concentration of potassium ions was not measured in each final reconstituted sample, conductivity was measured and can be used as an approximate surrogate measure. In samples that showed toxicity, the average conductivity was 873 : mhos, and in samples that did not show toxicity, the average conductivity was 797 : mhos. The differences in conductivity between tests that indicated toxicity and tests that did not were statistically significantly different (at the  $\alpha = 0.05$  level). This finding indicates that those samples which were less diluted in the reconstitution process, were also more likely to be toxic.

Several commenters also expressed concern over the way EPA handled outlier data points in the WET Interlaboratory Variability Study. EPA believes that outliers were treated according to standard practice and according to ASTM standards for measuring method precision. EPA identified outliers using ASTM's h and k statistics, and discarded outliers only when a probable cause for the outlier was identified. In all, only eight tests in the entire study of 698 tests were excluded based on outlier analysis.

### 3. Variability

Several commenters stated that the variability of the WET methods (measured in terms of CV) is too high for use in NPDES permits. Commenters also recommended that specific steps be taken to account for variability in the permit limit derivation and compliance determination process. EPA believes that the WET Interlaboratory Variability Study accurately estimated the precision of WET test methods, and that this precision is adequate for regulatory use of the WET methods. The precision measured for the WET test methods is comparable to that of chemical methods. While EPA agrees with commenters that WET test methods cannot be compared in all aspects to chemical methods, the comparison of interlaboratory precision values does demonstrate that WET test methods are no more variable than other methods approved at 40 CFR Part 136 and used for regulatory compliance purposes.

In a recent peer-reviewed guidance document (USEPA, 2000c), EPA thoroughly evaluated the issue of WET test method variability and accounting for such variability in NPDES applications. The document concluded that "comparisons of WET method precision with method precision for analytes commonly limited in NPDES permits clearly demonstrate that the variability of the promulgated WET methods is within the range of variability experienced in other types of [required regulatory] analyses." The analytical variability of WET test methods is

accounted for appropriately in the development of permit limits derived according to EPA's Technical Support Document (TSD) (USEPA, 1991). The TSD approach accounts for both effluent variability and method variability. The TSD statistical approach to determination of reasonable potential and permit limit derivation considers combined effluent and analytical variability through the CV of measured effluent values. Because the determination of effluent variability is based on empirical measurements, the variability estimated for effluent measurements includes the variability of pollutant levels, sampling variability, and a smaller component owed to method variability.

EPA does not recommend additional approaches or factors to account for variability, because the TSD approach appropriately accounts for method variability in the permit derivation process. In the guidance document, EPA evaluated additional approaches to account for variability in the permit derivation process and concluded that such approaches would not ensure adequate protection of water quality. The TSD approach was designed to provide a reasonable degree of protection for water quality as well as from effluent and analytical variability. Alternative approaches would undermine these objectives.

Some commenters expressed specific concern that the *Selenastrum capricornutum* Growth Test method was too variable. EPA believes that the variability of the *Selenastrum capricornutum* Growth Test method, as measured in the WET Interlaboratory Variability Study (USEPA, 2001a) and variability guidance document (USEPA, 2000c), is acceptable for the intended regulatory use of the methods. EPA observed in the WET Interlaboratory Variability Study that the variability of the *Selenastrum capricornutum* Growth Test method was lower when the method was conducted with the addition of EDTA. In today's action, EPA is removing the option to conduct the test without the addition of EDTA when data is submitted under NPDES

permits. EPA believes that this modification will improve the overall performance of the test method. False positive rates decreased from 33.3% to 0.00% and interlaboratory variability decreased from 58.5% to 34.3% when EDTA was added. EPA cautions, however, that the required addition of EDTA may make the *Selenastrum capricornutum* Growth Test less sensitive, thus less useful, for measuring the toxicity of some test samples, specifically, samples that contain toxic levels of metals.

#### 4. Successful Test Completion Rate

Some commenters stated that EPA incorrectly calculated successful test completion rates in the WET Interlaboratory Variability Study by failing to invalidate tests that did not meet specific test condition ranges. As previously discussed (see Section VI.C.2 of this preamble), EPA accurately invalidated tests according to the test acceptability criteria specific to each method, and successful test completion rates were based on meeting these criteria. EPA acknowledges that the promulgated methods allow flexibility in the review of test conditions. The method manuals state that departures in specified test condition ranges do not necessarily invalidate test results. In today's action EPA has modified the methods to better clarify this allowable flexibility. For the purposes of reviewing data submitted under NPDES permits, the manuals now clearly distinguish between requirements of the method and recommended test condition ranges.

Several commenters stated that the successful test completion rate measured for the *Ceriodaphnia dubia* Survival and Reproduction Test method was unacceptable and indicates a lack of ruggedness. EPA believes that the successful test completion rate observed for the *Ceriodaphnia dubia* Survival and Reproduction Test method in the WET Interlaboratory Variability Study was artificially suppressed by very poor performance in a small subset of laboratories. Only ten of the 34 participant laboratories performed invalid tests, but eight of these

laboratories performed invalid tests on 50% or more of the samples tested. The low rate of successful test completion in these eight laboratories may have been influenced by the Study's strict testing schedule, which required that each test be conducted on a given day and that all tests be conducted within a 15-day time period. When invalid tests conducted in a given laboratory were likely due to marginal or poor health of the test organism cultures, then it was logical that the laboratory would fail a high percentage of tests during the Study because culture health was unlikely to fully recover within 15 days. EPA believes that measuring an individual laboratory's rate of successful test completion over a 15-day period may not be representative of that laboratory's overall successful test completion rate. For instance, several laboratories had successful test completion rates of 0% during the WET Interlaboratory Variability Study. Obviously, this result is not indicative of the laboratory's overall successful test completion rate. If so, the laboratory would not be in business or would not have been able to prequalify for participation in the Study. EPA believes that successful test completion rates for this method are higher in routine use because testing laboratories are allowed flexibility in the timing of sample collection and can avoid initiating tests during periods of marginal to poor culture health.

Some commenters expressed concern that the successful test completion rate for the *Selenastrum capricornutum* Growth Test method was too low. In today's action, EPA is removing the option to conduct the test without the addition of EDTA. EPA believes that this modification will improve successful test completion rates for the method as laboratories consistently culture and test with EDTA. The successful test completion rate of 63.6% (when conducted with EDTA) was in part due to laboratory inexperience in using both the with and without-EDTA techniques. For example, two laboratories that cultured organisms without EDTA and generally conducted tests without EDTA showed poor successful test completion rates

(failing eight of eight tests) when EDTA was used. These laboratories failed all eight tests conducted with EDTA and passed all but one test (seven of eight) without EDTA. Commenters point out that laboratories were prequalified for participation in the WET Interlaboratory Variability Study, but this prequalification required only experience with the method, not experience with both the with and without-EDTA procedures of the method. Some laboratories cultured organisms and typically conducted tests with EDTA, and other laboratories cultured organisms and typically conducted tests without EDTA.

#### 5. False Positive Rate

Several comments stated that EPA underestimated the false positive rates measured in the WET Interlaboratory Variability Study and that the measured rates are unacceptably high for regulatory use. In the context of WET methods, the false positive rate is the rate at which tests conducted on non-toxic dilution waters indicate the presence of toxicity (i.e., NOEC, LC50, or IC25 test endpoints are <100% effluent). EPA disagrees with comments that stated that false positive rates for WET test methods are unacceptably high. EPA's WET Interlaboratory Variability Study conclusively showed that measured false positive rates were below the theoretical rate of 5% estimated for the methods. Measured false positive rates were 3.7% for the *Ceriodaphnia dubia* Survival and Reproduction Test method, 4.35% for the Fathead Minnow Larval Survival and Growth Test method, and 0% for all other methods evaluated in the WET Interlaboratory Variability Study (with the exception of the *Selenastrum capricornutum* Growth Test conducted without EDTA, which EPA is removing as an option in today's action). A total of 150 valid WET tests were conducted on blank samples in the Study. Of these, only two tests (1.3%) resulted in a false positive result.

The WET Interlaboratory Variability Study conclusively demonstrated that the false positive rate of WET methods is at or below the level expected for the methods. While this rate is low (below 5%), false positives do occur. EPA accounts for this possibility in the compliance and enforcement guidance. EPA policy states that “EPA does not recommend that the initial response to a single exceedance of a WET limit, causing no known harm, be a formal enforcement action with a civil penalty” (USEPA, 1995a). EPA policy suggests additional testing is an appropriate initial response to a single WET limit exceedance.

Several commenters expressed concern that WET tests do not have method detection limits as contained in chemical methods to protect from reporting false positive results. As previously discussed (see Section VI.C.1 of this preamble), method detection limit concepts are not applicable to WET test methods and have not been applied historically to toxicity testing methods developed by EPA or by voluntary consensus standards bodies.

EPA established the method detection limit (MDL) concept specifically for chemical methods, where results generally consist of a single measurement of the pollutant of interest by an analytical instrument. The MDL concept uses information about the variability of the measurement system to determine a response level at which the measurement can be reliably distinguished from background “noise,” thus providing protection from false positive results. In WET testing, the final result is not based on a single measurement, but is the product of a series of replicated measurements on a range of effluent concentrations. The additional measurements, controls, replication, and statistical approaches included in the WET test method “measurement system” ensure that measured responses can be reliably distinguished from background noise.

While results from chemical methods may rely on a single instrument measurement, each WET test is designed as an experiment. WET tests contain at least six treatments, each replicated

from four to ten times. Measurements are made on each replicate of each treatment, so that results reflect average responses and the variability of those responses can be estimated. Each test also includes a control treatment, which is also replicated. This control treatment provides a measure of the background response and the “noise” or variability associated with that response.

The control response is then compared to the response in effluent treatments using statistical methods to test the hypothesis that treatments containing effluent are not significantly different from the control treatment. If this hypothesis is rejected (considering the measured background or control responses, the treatment responses, and the variability associated with those responses), then the effluent is considered toxic. Hypothesis testing techniques provide protection from false positive results by specifically setting the Type I error rate allowed in rejecting the null hypothesis. Point estimation techniques use regression analysis to determine the effluent concentration that produces a specified level of response (e.g., the IC25 endpoint specifies a 25% difference between control and effluent treatment response in order for the effluent to be determined as toxic). In this case, false positive protection is inherently provided by the level of response required for generation of the selected endpoint. EPA believes that the test design employed in WET testing (including controls, replication, and hypothesis testing or point estimation) provides adequate protection from false positives.

## 6. Implementation

Some commenters commented on issues specifically related to the implementation of WET permits, such as reasonable potential determinations, independent applicability of WET limits, discharge monitoring report certifications, and use of WET methods in NPDES permits. Many such comments are beyond the scope of this rulemaking. In the proposed rulemaking, EPA invited comments “only on the conduct of WET test methods and not on the implementation of



WET control strategies through NPDES permits.” EPA recognizes that NPDES permittees have continuing concerns about implementation of WET requirements in NPDES permits. In a 'WHEREAS clause' to the Settlement Agreement described previously, EPA acknowledged that the provisions of the Settlement Agreement, which focused primarily on test methodology and, to a lesser extent, interpretation of test results, did not address all of the litigants' concerns regarding applicability of WET testing requirements to particular waterbodies (with specific reference to intermittent or effluent dependent waterbodies located in the Arid West) and did not address many of the litigants' concerns regarding regulatory implementation of WET control programs (e.g., toxicity identification evaluation requirements, toxicity reduction evaluation requirements, compliance determinations, and trigger thresholds). In addition, the Settlement Agreement also acknowledged that the 1995 rule, which incorporated the WET test methods in dispute, did not specify means to adjust for the frequency, duration, or magnitude of instream exposure conditions, and that such decisions are to be made by the regulatory authority in the context of water quality standard setting and/or NPDES permitting decisions. EPA continues to acknowledge these continuing concerns and will continue to address implementation concerns as they arise in concrete circumstances or through guidance, as appropriate.

## **VII. Statutory and Executive Order Reviews**

### *A. Executive Order 12866: Regulatory Planning and Review*

Under Executive Order 12866 (58 FR 51735; October 4, 1993), the Agency must determine whether the regulatory action is "significant" and therefore subject to Office of Management and Budget (OMB) review and the requirements of the Executive Order. The Executive Order defines "significant regulatory action" as one that is likely to result in a rule that may:

(1) Have an annual effect on the economy of \$100 million or more, or adversely affect in a material way the economy, a sector of the economy, productivity, competition, jobs, the environment, public health or safety, or State, local, or Tribal governments or communities;

(2) Create a serious inconsistency or otherwise interfere with an action taken or planned by another agency;

(3) Materially alter the budgetary impact of entitlements, grants, user fees, or loan programs or the rights and obligations of recipients thereof; or

(4) Raise novel legal or policy issues arising out of legal mandates, the President's priorities, or the principles set forth in the Executive Order.

It has been determined that this rule is not a "significant regulatory action" under the terms of Executive Order 12866 and is therefore not subject to OMB review.

#### *B. Paperwork Reduction Act*

This action does not impose an information collection burden under the provisions of the Paperwork Reduction Act, 44 U.S.C. 3501 *et seq.* This rule revises and ratifies test methods that are currently approved for use in NPDES permits and does not impose any additional information collection requirements.

Burden means the total time, effort, or financial resources expended by persons to generate, maintain, retain, or disclose or provide information to or for a Federal agency. This includes the time needed to review instructions; develop, acquire, install, and utilize technology and systems for the purposes of collecting, validating, and verifying information, processing and maintaining information, and disclosing and providing information; adjust the existing ways to comply with any previously applicable instructions and requirements; train personnel to be able to respond to a

collection of information; search data sources; complete and review the collection of information; and transmit or otherwise disclose the information.

An Agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a currently valid OMB control number. The OMB control numbers for EPA's regulations are listed in 40 CFR Part 9 and 48 CFR Chapter 15.

### *C. Regulatory Flexibility Act*

The Regulatory Flexibility Act (RFA) generally requires an agency to prepare a regulatory flexibility analysis of any rule subject to notice and comment rulemaking requirements under the Administrative Procedure Act or any other statute unless the agency certifies that the rule will not have a significant economic impact on a substantial number of small entities. Small entities include small businesses, small organizations, and small governmental jurisdictions.

For purposes of assessing the impacts of today's rule on small entities, small entity is defined as: (1) a small business as defined by the U.S. Small Business Administration definitions at 13 CFR 121.201; (2) a small governmental jurisdiction that is a government of a city, county, town, school district or special district with a population of less than 50,000; and (3) a small organization that is any not-for-profit enterprise which is independently owned and operated and is not dominant in its field.

After considering the economic impacts of today's final rule on small entities, I certify that this action will not have a significant economic impact on a substantial number of small entities. Today's rule revises and ratifies EPA WET test methods currently approved for use at 40 CFR Part 136. Overall, the costs of these revisions are minimal. While some of the revisions may increase costs (e.g., quality control requirements), EPA believes that these costs will be alleviated by a potential reduction in retesting and additional investigations (e.g., accelerated testing,

toxicity identification evaluations, or toxicity reduction evaluations) by the permittee that may result from improved test performance and increased confidence in the reliability of testing results. Many of the laboratories that conduct WET testing are already implementing the additional requirements, further minimizing any potential cost increases. EPA estimates that the average incremental cost per permit per year for today's method revisions is \$276. Because monitoring frequency is typically less frequent for small entities than large entities, EPA expects the average incremental cost per permit per year to be even less than \$276 for small entities. Using a cost of \$276 and average revenue information for small governmental jurisdictions and businesses, EPA estimates that the incremental costs for these method revisions are less than 0.1 percent of revenue for small entities.

#### *D. Unfunded Mandates Reform Act*

Title II of the Unfunded Mandates Reform Act of 1995 (UMRA), Public Law 104-4, establishes requirements for Federal agencies to assess the effects of their regulatory actions on State, Tribal, and local governments and the private sector. Under section 202 of the UMRA, EPA generally must prepare a written statement, including a cost-benefit analysis, for proposed and final rules with “Federal mandates” that may result in expenditures to State, Tribal, and local governments, in the aggregate, or to the private sector, of \$100 million or more in any one year. Before promulgating an EPA rule for which a written statement is needed, section 205 of the UMRA generally requires EPA to identify and consider a reasonable number of regulatory alternatives and adopt the least costly, most cost-effective or least burdensome alternative that achieves the objectives of the rule. The provisions of section 205 do not apply when they are inconsistent with applicable law. Moreover, section 205 allows EPA to adopt an alternative other

than the least costly, most cost-effective or least burdensome alternative if the Administrator publishes with the final rule an explanation of why that alternative was not adopted.

Before EPA establishes any regulatory requirements that may significantly or uniquely affect small governments, including Tribal governments, it must have developed under section 203 of the UMRA a small government agency plan. The plan must provide for the notification of potentially affected small governments, enabling officials of affected small governments to have meaningful and timely input in the development of EPA regulatory proposals with significant Federal intergovernmental mandates, and informing, educating, and advising small governments on compliance with the regulatory requirements.

EPA has determined that today's rule does not contain a Federal mandate that may result in expenditures of \$100 million or more for State, Tribal, and local governments, in the aggregate, or the private sector in any one year. This rule promulgates revisions to WET test methods that are currently approved for use in NPDES permits and certification of Federal licenses under the CWA. The revisions are minor and the cost to implement them is minimal. Thus, today's rule is not subject to sections 202 and 205 of the UMRA. For the same reasons, EPA has also determined that this rule contains no regulatory requirements that might significantly or uniquely affect small governments. Thus, today's rule also is not subject to the requirements of section 203 of the UMRA.

#### *E. Executive Order 13132: Federalism*

Executive Order 13132, entitled "Federalism" (64 FR 43255; August 10, 1999), requires EPA to develop an accountable process to ensure "meaningful and timely input by State and local officials in the development of regulatory policies that have federalism implications." "Policies that have federalism implications" is defined in the Executive Order to include regulations that

have “substantial direct effects on the States, on the relationship between the national government and the States, or on the distribution of power and responsibilities among the various levels of government.”

This final rule does not have federalism implications. It will not have substantial direct effects on the States, on the relationship between the national government and the States, or on the distribution of power and responsibilities among the various levels of government, as specified in Executive Order 13132. Today’s rule promulgates revisions to WET test methods that are currently approved for use in NPDES permits and certification of Federal licenses under the CWA. The revisions are minor and the cost to implement them is minimal. Thus, Executive Order 13132 does not apply to this rule.

*F. Executive Order 13175: Consultation and Coordination with Indian Tribal Governments*

Executive Order 13175, entitled “Consultation and Coordination with Indian Tribal Governments” (65 FR 67249; November 9, 2000), requires EPA to develop an accountable process to ensure “meaningful and timely input by Tribal officials in the development of regulatory policies that have Tribal implications.” “Policies that have Tribal implications” is defined in the Executive Order to include regulations that have “substantial direct effects on one or more Indian Tribes, on the relationship between the Federal government and the Indian Tribes or on the distribution of power and responsibilities between the Federal government and Indian Tribes.”

This final rule does not have Tribal implications. It will not have substantial direct effects on Tribal governments, on the relationship between the Federal government and Indian Tribes, or on the distribution of power and responsibilities between the Federal government and Indian Tribes, as specified in Executive Order 13175. Today’s rule promulgates revisions to WET test methods

that are currently approved for use in NPDES permits and certification of Federal licenses under the CWA. The revisions are minor and the cost to implement them is minimal. Thus, Executive Order 13175 does not apply to this rule.

*G. Executive Order 13045: Protection of Children From Environmental Health and Safety Risks*

Executive Order 13045 (62 FR 19885; April 23, 1997) applies to any rule that: (1) is determined to be “economically significant” as defined under Executive Order 12866, and (2) concerns an environmental health or safety risk that EPA has reason to believe may have a disproportionate effect on children. If the regulatory action meets both criteria, the Agency must evaluate the environmental health or safety effects of the planned rule on children, and explain why the planned regulation is preferable to other potentially effective and reasonably feasible alternatives considered by the Agency. This rule is not subject to the Executive Order because it is neither “economically significant” as defined in Executive Order 12866, nor does it concern an environmental health or safety risk that EPA has reason to believe may have a disproportionate effect on children.

*H. Executive Order 13211: Actions Concerning Regulations that Significantly Affect Energy Supply, Distribution, or Use*

This rule is not subject to Executive Order 13211, “Actions Concerning Regulations That Significantly Affect Energy Supply, Distribution, or Use” (66 FR 28355; May 22, 2001) because it is not a significant regulatory action under Executive Order 12866.

*I. National Technology Transfer and Advancement Act*

Section 12(d) of the National Technology Transfer and Advancement Act of 1995, (“NTTAA”), Public Law 104-113, section 12(d) (15 U.S.C. 272 note), directs EPA to use voluntary consensus standards in its regulatory activities unless to do so would be inconsistent

with applicable law or otherwise impractical. Voluntary consensus standards are technical standards (e.g., material specifications, test methods, sampling procedures, business practices) that are developed or adopted by voluntary consensus standards bodies (VCSBs). The NTTAA directs EPA to provide Congress, through the Office of Management and Budget (OMB), explanations when the Agency decides not to use available and applicable voluntary consensus standards.

This rulemaking would revise existing EPA WET test methods. For the methods that EPA is revising, the Agency did not conduct a search to identify potentially applicable voluntary consensus standards, because the revisions EPA is promulgating today would merely incorporate more specificity and detail into currently approved EPA test methods. EPA did, however, consult available voluntary consensus standards, such as ASTM standards, for guidance in conducting the Interlaboratory Variability Study and in defining certain performance characteristics of the methods.

#### *J. Congressional Review Act*

The Congressional Review Act, 5 U.S.C. 801 et seq., as added by the Small Business Regulatory Enforcement Fairness Act of 1996 (SBREFA), generally provides that before a rule may take effect, the agency promulgating the rule must submit a rule report, which includes a copy of the rule, to each House of the Congress and to the Comptroller General of the United States. EPA will submit a report containing this rule and other required information to the U.S. Senate, the U.S. House of Representatives, and the Comptroller General of the United States prior to publication of the rule in the *Federal Register*. A major rule cannot take effect until 60 days after it is published in the *Federal Register*. This action is not a “major rule” as defined by 5 U.S.C. 804(2). This rule will be effective on **[Insert 30 days from publication date in the**



**Federal Register.]**

## **VIII. References**

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Toxicity Enforcement, July 6, 1995, memorandum from Brian Maas, U.S. Environmental Protection Agency, Office of Enforcement and Compliance Assurance, Washington D.C.

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U.S. Environmental Protection Agency. 2000b. Notice of availability, "Understanding and accounting for method variability in WET applications under the NPDES program." 65 FR 44528-44529.

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FR 58693-58694.

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Technology, Washington, D.C.

Waller, T.W., L.P. Ammann, W.J. Birge, K.L. Dickson, P.B. Dorn, N.E. LeBlanc, D.I. Mount, B.R. Parkhurst, H.R. Preston, S.C. Schimmel, A. Spacie, and G.B. Thursby. 1996. Discussion synopsis, predicting instream effects from WET tests. Chapter 9 In: *Whole Effluent Toxicity Testing: An Evaluation of Methods and Prediction of Receiving System Impacts*. D.R. Grothe, K.L. Dickson, and D.K. Reed-Judkins, eds., SETAC Press, Pensacola,

FL, pp. 271-286.

### **List of Subjects at 40 CFR Part 136**

Environmental protection, Incorporation by reference, Reporting and recordkeeping requirements, Water pollution control.

Dated: \_\_\_\_\_

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**Christine Todd Whitman,**

*Administrator.*

For the reasons set out in the preamble, title 40, chapter I of the Code of Federal Regulations, is amended as follows:

### **PART 136 - GUIDELINES ESTABLISHING TEST PROCEDURES FOR THE ANALYSIS OF POLLUTANTS**

1. The authority citation for Part 136 continues to read as follows:

**Authority:** Secs. 301, 304(h), 307, and 501(a), Pub. L. 95-217, 91 Stat. 1566, *et seq.* (33 U.S.C. 1251, *et seq.*) (The Federal Water Pollution Control Act Amendments of 1972 as amended by the Clean Water Act of 1977).

2. Section 136.3 is amended:

a. In Table IA of paragraph (a) by revising entries 6 to 9.

- b. In paragraph (b) by revising references (34), (38), and (39).
- c. In paragraph (b) by removing and reserving reference (42).

The revisions read as follows:

§136.3 Identification of test procedures.

(a) \* \* \*

TABLE IA.—LIST OF APPROVED BIOLOGICAL METHODS

Parameter and units	Method <sup>1</sup>	EPA	Standard Methods 18 <sup>th</sup> , 19 <sup>th</sup> , 20 <sup>th</sup> Ed.	ASTM	AOAC	USGS	Other
* * * * *							
Aquatic Toxicity:							
6. Toxicity, acute, fresh water organisms, LC50, percent effluent.	<i>Ceriodaphnia dubia</i> acute	2002.0 <sup>7</sup>					
	<i>Daphnia pulex</i> and <i>Daphnia magna</i> acute	2021.0 <sup>7</sup>					
	Fathead minnow, <i>Pimephales promelas</i> , and Bannerfin shiner, <i>Cyprinella leedsii</i> , acute	2000.0 <sup>7</sup>					
	Rainbow trout, <i>Oncorhynchus mykiss</i> , and brook trout, <i>Salvelinus fontinalis</i> , acute	2019.0 <sup>7</sup>					
7. Toxicity, acute, estuarine and marine organisms of the Atlantic Ocean and Gulf of Mexico, LC50, percent effluent.	Mysid, <i>Mysidopsis bahia</i> , acute	2007.0 <sup>7</sup>					
	Sheepshead minnow, <i>Cyprinodon variegatus</i> , acute	2004.0 <sup>7</sup>					
	Silverside, <i>Menidia beryllina</i> , <i>Menidia menidia</i> , and <i>Menidia peninsulae</i> , acute	2006.0 <sup>7</sup>					
8. Toxicity, chronic, fresh water organisms, NOEC or IC25, percent effluent.	Fathead minnow, <i>Pimephales promelas</i> , larval survival and growth	1000.0 <sup>8</sup>					
	Fathead minnow, <i>Pimephales promelas</i> , embryo-larval survival and teratogenicity	1001.0 <sup>8</sup>					
	Daphnia, <i>Ceriodaphnia dubia</i> , survival and reproduction	1002.0 <sup>8</sup>					
	Green alga, <i>Selenastrum capricornutum</i> , growth	1003.0 <sup>8</sup>					



Parameter and units	Method <sup>1</sup>	EPA	Standard Methods 18 <sup>th</sup> , 19 <sup>th</sup> , 20 <sup>th</sup> Ed.	ASTM	AOAC	USGS	Other
9. Toxicity, chronic, estuarine and marine organisms of the Atlantic Ocean and Gulf of Mexico, NOEC or IC25, percent effluent.	Sheepshead minnow, <i>Cyprinodon variegatus</i> , larval survival and growth	1004.0 <sup>9</sup>					
	Sheepshead minnow, <i>Cyprinodon variegatus</i> , embryo-larval survival and teratogenicity	1005.0 <sup>9</sup>					
	Inland silverside, <i>Menidia beryllina</i> , larval survival and growth						
	Mysid, <i>Mysidopsis bahia</i> , survival, growth, and fecundity	1006.0 <sup>9</sup>					
	Sea urchin, <i>Arbacia punctulata</i> , fertilization	1007.0 <sup>9</sup>					
		1008.0 <sup>9</sup>					

Notes to Table IA:

<sup>1</sup> The method must be specified when results are reported.

\* \* \* \* \*

<sup>7</sup> USEPA. October 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fifth Edition. U.S. Environmental Protection Agency, Office of Water, Washington, D.C. EPA 821-R-02-012.

<sup>8</sup> USEPA. October 2002. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Fourth Edition. U.S. Environmental Protection Agency, Office of Water, Washington, D.C. EPA 821-R-02-013.

<sup>9</sup> USEPA. October 2002. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. Third Edition. U.S. Environmental Protection Agency, Office of Water, Washington, D.C. EPA 821-R-02-014.

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(b) \* \* \*

#### REFERENCES, SOURCES, COSTS, AND TABLE CITATIONS:

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(34) USEPA. October 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fifth Edition. U.S. Environmental Protection Agency, Office of Water, Washington, D.C. EPA 821-R-02-012. Available from: National Technical Information Service, 5285 Port Royal Road, Springfield, Virginia 22161, Publ. No. PB2002-108488. Table IA, Note 7.

\* \* \* \* \*

(38) USEPA. October 2002. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Fourth Edition. U.S. Environmental Protection Agency, Office of Water, Washington, D.C. EPA 821-R-02-013. Available from: National Technical Information Service, 5285 Port Royal Road, Springfield, Virginia 22161, Publ. No. PB2002-108489. Table IA, Note 8.

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(42) [RESERVED]

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